

## Quantitative Analysis by Diffuse Reflectance Infrared Fourier Transform and Linear Stepwise Multiple Regression Analysis I —Simultaneous quantitation of ethenzamide, isopropylantipyrine, caffeine, and allylisopropylacetylurea in tablet by DRIFT and linear stepwise multiple regression analysis—

**ManKi Park, HyeRan Yoon, KyoungHo Kim and JungHwan Cho**  
*Department of Pharmacy, College of Pharmacy, Seoul National University,  
Seoul 151-742, Korea  
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**Abstract** □ Quantitation of ethenzamide, isopropylantipyrine and caffeine takes about 41 hrs by conventional GC method. Quantitation of allylisopropylacetylurea takes about 40 hrs by conventional UV method. But quantitation of them takes about 6 hrs by DRIFT developing method. Each standard and sample sieved, powdered and acquired DRIFT spectrum. Out of them peak of each component was selected and ratio of each peak to standard peak was acquired, and then linear stepwise multiple regression was performed with these data and concentration. Reflectance value, Kubelka-Munk equation and Inverse-Kubelka-Munk equation were modified by us. Inverse-Kubelka-Munk equation completed the deficit of Kubelka-Munk equation. Correlation coefficients acquired by conventional GC and UV against DRIFT were more than 0.95.

**Keywords** □ quantitation, DRIFT, linear stepwise multiple regression analysis, Kubelka-Munk equation

Infrared spectrophotometry has been mainly used in identification of material, determination of functional group in qualitative analysis, and auxiliary a way in quantitative analysis. The possibility of carrying out the quantitative analysis entirely on the basis of infrared spectrophotometry reexamined.

In this study the experiment was performed by linking FT-IR with diffuse reflectance spectra (DRS), a useful new technique known as diffuse reflectance fourier transform infrared spectrometry (DRIFT)<sup>1-16</sup>. The author will demonstrate that this technique provides a powerful method for quick and reliable solid-form dosage analysis.

Least-squares method<sup>17-20</sup> employed in this study offered the advantages that they could be applied under conditions of (1) complete spectral overlap, (2) signal-to-noise ratio of less than one, (3) nonzero and/or nonlinear baseline and (4) failure of the Beer-Lambert relation in a portion of the spectrum. Also, this study presents here Linear Stepwise Multiple Regression (LSMR) analysis<sup>21-24</sup> which use all the important spectral information contained in a set of standard spectra consisting of

mixtures of known multi-component concentrations. The advantages of these LSMR will be demonstrated for the quantitative analysis of spectra measured from real samples.

The technique of DRS is concerned with the efficient collection of diffusely scattered light, at the direction unrelated to that of the incident radiation (Fig. 1).

For interpretation, the Kubelka-Munk analysis, sometimes called "the Beer's law of reflectance spectroscopy" is often used. As will be known, the Beer-Lambert law is a limiting law which is obeyed only in diluted solutions. The Kubelka-Munk function should also be regarded as a limiting law for essentially the same reason<sup>9,25</sup>.

Several theories have been proposed for the quantitative interpretation of the diffuse reflectance spectra. The Kubelka-Munk (K-M) equation relationship<sup>7,13,26</sup> is the equation most commonly used to describe the reflectance from a scattering medium.

The reflectance is related to the absorption constant K and scattering constant S of the mixture of the sample and medium. Thus, the K-M equation

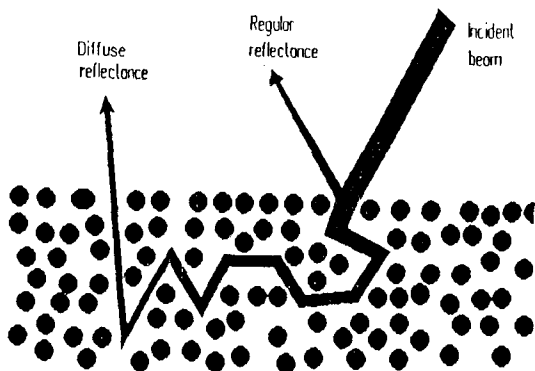


Fig. 1. Diagrammatic depiction of Diffuse reflectance.

can be written as

$$f(R) = \frac{(1-R)^2}{2R} = \frac{K}{S}, \quad (\text{eq. 1})$$

where  $f(R)$  is the K-M function and  $R$  the absolute reflectance.

In practice, the relative reflectance  $r$  is used in place of  $R$  in eq. 1. The relation between relative reflectance and concentration varies with dispersing medium. The following assumptions are usually made. The absorption constant  $K$  is equal to the product of the absorption coefficient and the concentration  $X$  of the sample, and the scattering constant  $S$  is equal to the scattering coefficient of the scattering medium.

Thus, the equation is

$$f(r) = \frac{(1-r)^2}{2r} = \frac{\epsilon s X}{\sigma m}, \quad (\text{eq. 2})$$

The relation follows the K-M equation only in the low concentration region. The deviation in high concentration region from the K-M equation changes with wavelength and dispersing medium. The result over the whole concentration range can be interpreted by means of an inverse K-M equation. The factors determining the intensity of absorption band are not only the absorption coefficient but also the scattering coefficient of the sample. Both constants would depend on the concentration of both sample and dispersion medium. Thus, the K-M equation should be written as

$$f(r) = \frac{(1-r)^2}{2r} = \frac{\epsilon s X + \epsilon m (1-X)}{\sigma s X + \sigma m (1-X)}, \quad (\text{eq. 3})$$

where  $\sigma s$  and  $\sigma m$  are the scattering coefficients of sample and medium,  $\epsilon s$  and  $\epsilon m$  the absorption coefficients of the sample and dispersion medium, respectively. The absorption coefficient of the dispersion medium has no characteristic absorp-

tion. Thus, the equation is

$$f(r) = \frac{(1-r)^2}{2r} = \frac{\nu X}{1 + (\delta - 1)X}, \quad (\text{eq. 4})$$

where  $\delta$  is the ratio of scattering coefficient of the sample to that of the dispersing medium,  $\sigma s/\sigma m$ , and  $\nu$  is the ratio of the absorption coefficient of the sample to the scattering coefficient of the dispersing medium,  $\epsilon s/\sigma m$ .

Finally, the inverse K-M equation should be written as

$$\frac{1}{f(r)} = \frac{\delta - 1}{\nu} + \frac{1}{\nu X} \quad (\text{eq. 5})$$

In the inverse K-M equation, the inverse K-M function is inversely proportional to the concentration.

The estimated standard mixture spectra generated by DRIFT described here could be good use in calculating the content of four-component. And then, the actual estimated sample concentrations were obtained via inverse K-M equation.

This applicability of inverse K-M equation to spectrometer data via the regression matrix approach will be shown here. Correlation data with deviation from K-M equation in low concentration region will be examined, and comparisons for other analytical method will be made.

## MATERIALS AND METHODS

### Measurement of diffuse reflectance spectra

Perkin Elmer 1710 Fourier Transform Infrared Spectrophotometer equipped with Diffuse Reflectance Accessory and Model 3600 Data Station was used. These equipment were connected to Perkin Elmer Model PP-1 Plotter Printer. Fifty "scan" (interferograms) were accumulated at resolution of  $4\text{cm}^{-1}$  to obtain each spectrum. Each spectrum was stored on magnetic tape. Infrared spectra were detected with FR-DTGS (deuterated triglycine sulfate). Potassium bromide crystals for FT-IR were ground to fine powder and used as dispersing medium.

In a sample, fine powder of commercial tablet was used without further purification. Standard, sample and potassium bromide were prepared to fine powder and filtered through sieve (500 mesh).

The following procedure was performed for sample preparation.

- 1) 500mg of KBr was weighed out and placed in a jade mortar.
- 2) 10mg of sample (and/or standard) was weighed out accurately.
- 3) Sample (and/or standard) was placed in a

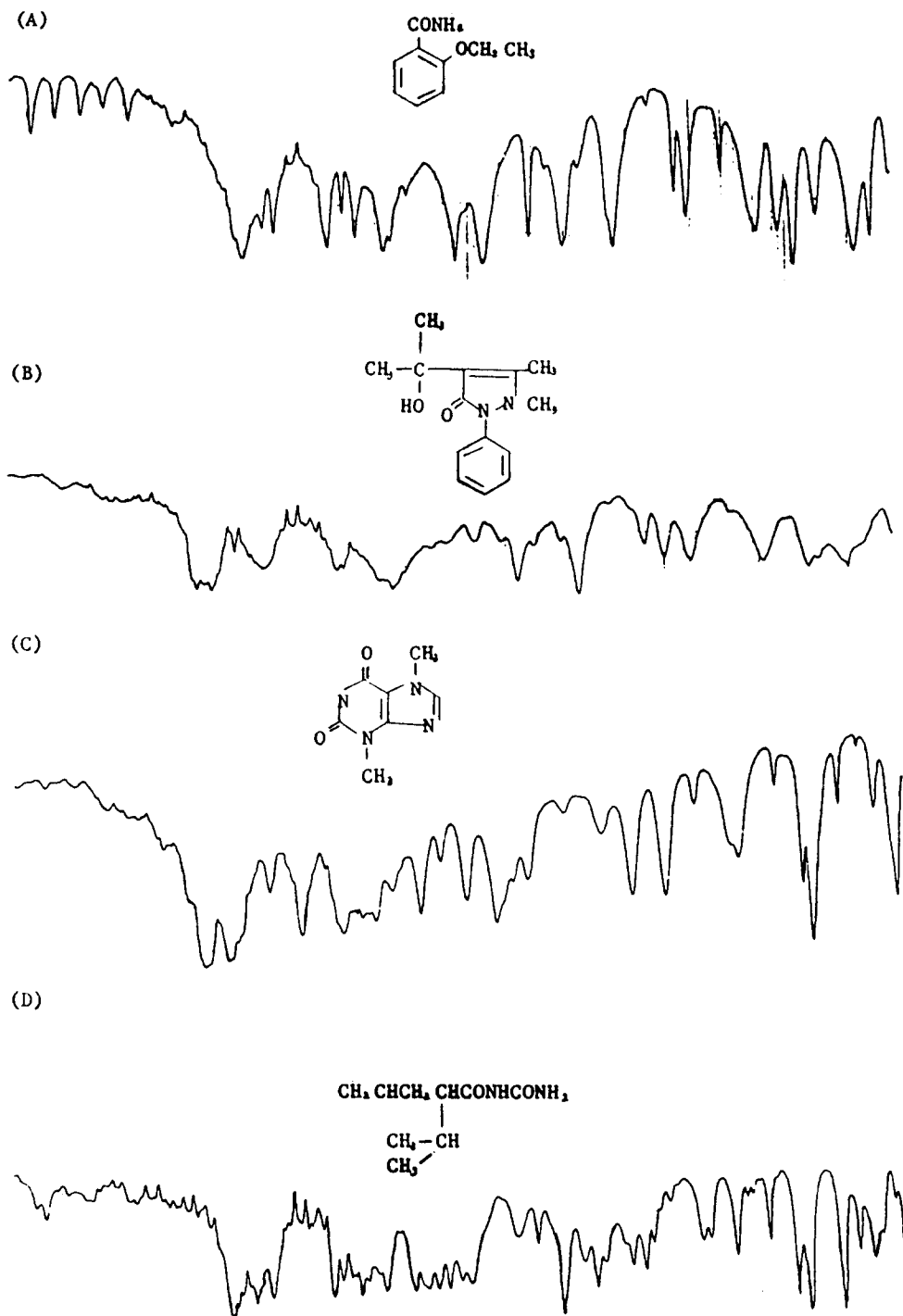


Fig. 2. Spectra of ethenzamide (A), isopropylantipyrine (B), caffeine (C) and allylisopropylacetylurea (D).

jade mortar, too.

- 4) They were ground for 10 min in a jade mortar with a pestle.
- 5) The ground sample was allowed to stand overnight in a desiccator before transferring into the DRIFT cell and recording the spectra.
- 6) Sample (and/or standard) was placed in a DRIFT cell.
- 7) DRIFT spectrum was measured (Fig. 2).

Procedure was described in Scheme 1.

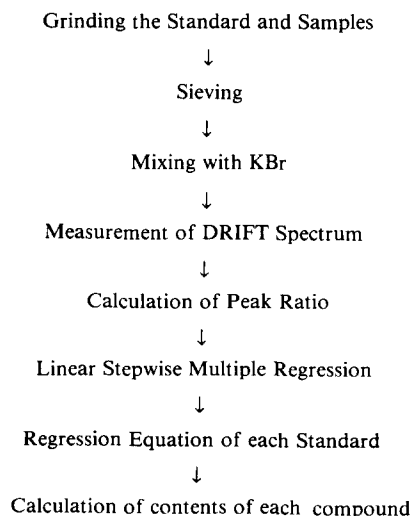
**Process for regression equation by linear stepwise multiple regression**

Ratio of each peak to reference peak was calculated after selection of nineteen peaks of four-component. "Multiple" means fitting the response, using more than two predictors. The basic idea of the method is to perform a regression with several variables as a series of straight line regressions.

LSMR was performed with experimental data against concentration. First, reflectance value itself was used by regression operation. Second, reflectance data was manipulated by K-M equation before regression. Third, reflectance data was manipulated by inverse K-M equation.

This treatment of data was applied in the development of method for the analysis of multi-component commercial tablet. Regression equation was calculated for each data manipulation and this was used for the quantitation of samples using predescribed equation. The result was compared with contents acquired by GC and UV.

Computational method for stepwise regression was described as follows:



**Scheme 1. Experimental scheme for DRIFT**

- 1) Construction of correlation matrix from the raw data
- 2) Stepwise procedure
  - a) Selection of the first variable to enter regression
  - b) The correlation matrix must be adjusted for the entrance variables into regression
  - c) Summary of information after a)-b)
  - d) Test for the elimination of variables already in regression
  - e) Selection of the next variable to enter regression
  - f) Creation of second variable to enter regression
  - g) Test for elimination of variables
  - h) Test for accepting new variable
  - i) Creation of third variable to enter regression
  - j) Test for elimination of variables
  - k) Test for accepting new variable

A least-square treatment of spectrometric data yielded equations for calculating concentrations of each four-component in standard mixtures. And then reflectances were measured at nineteen wavelength. Regression coefficients could be derived by this treatment. However, because numerical values for the coefficients was unnecessary, the final equation could be derived from directly.

Gas Chromatogram and calibration curve were presented in figures 3 and 4.

UV Calibration curve was described in figure 5.

## RESULT AND DISCUSSION

### *Selection of mixing ratio of dispersing medium and sample*

Fig. 6 shows the various mixing ratios of the sample and dispersing medium. In the case of a small amount of sample, selection of the absorption band was too small to find, whereas in a large amount of sample, the spectra was too wide in range to select the absorption band. Thus, mixing ratio of sample to dispersing medium for favorable spectrum in which was't under the influence of physico-chemical interaction<sup>27)</sup> is chosen 1 to 50.

### *Selection of nineteen peaks out of four-components*

The selection of the analytical band is perhaps one of the most important aspects of quantitative infrared analysis. Nevertheless, it is probably the aspect given the least consideration. Without proper selection of the analytical band, it is not possible to obtain accurate quantitative data. The analytical band selected should be very sensitive to small changes in the concentration of the com-

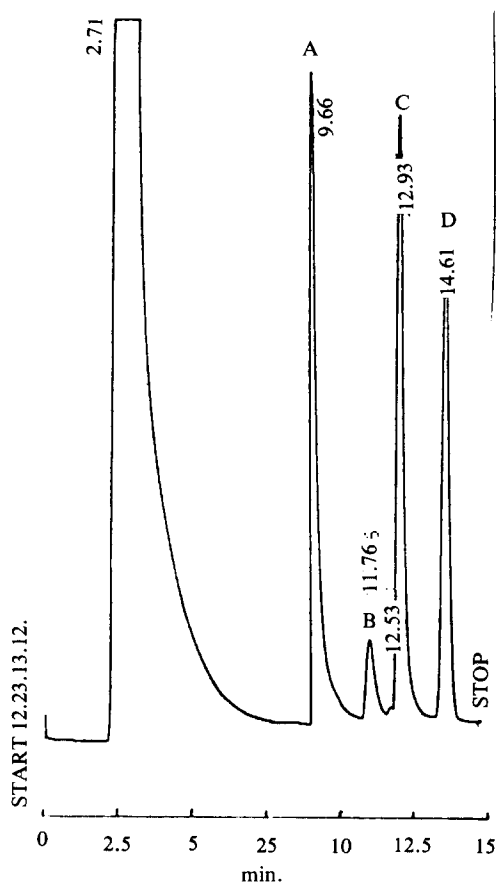


Fig. 3. Gas Chromatogram of ethenzamide (A), caffeine (B), isopropylantipyrene (C), and methylstearate (D).

Condition of GC

- o Instrument: Shimazu GC RIA
- o Column: 4.6mm i.d.  $\times$  2m long glass column with 3% OV-1 on Chromosorb WAW
- o Temperature
  - injection: 270°
  - column oven: 165°  $\xrightarrow{15^\circ}$  250°
  - detector: 270°
- o Detector: FID
- o Carrier gas: N<sub>2</sub>
- o Flow rate: 50ml/min.

pound being quantitated.

Regions of the spectrum where there are strong atmospheric absorption bands due to water vapor and carbon dioxide should be avoided. Thus, out of the peaks of spectrum obtained were chosen 19 peaks between 2000 and 400  $\text{cm}^{-1}$ . For example component ETH has an absorption bands at 1450 $\text{cm}^{-1}$ , attributed to the  $-\text{CH}_2$  bending and 1405 $\text{cm}^{-1}$ , due to  $-\text{CH}_3$  bending, at 1244 $\text{cm}^{-1}$ , due to aromatic ether and at 632 $\text{cm}^{-1}$ , due to

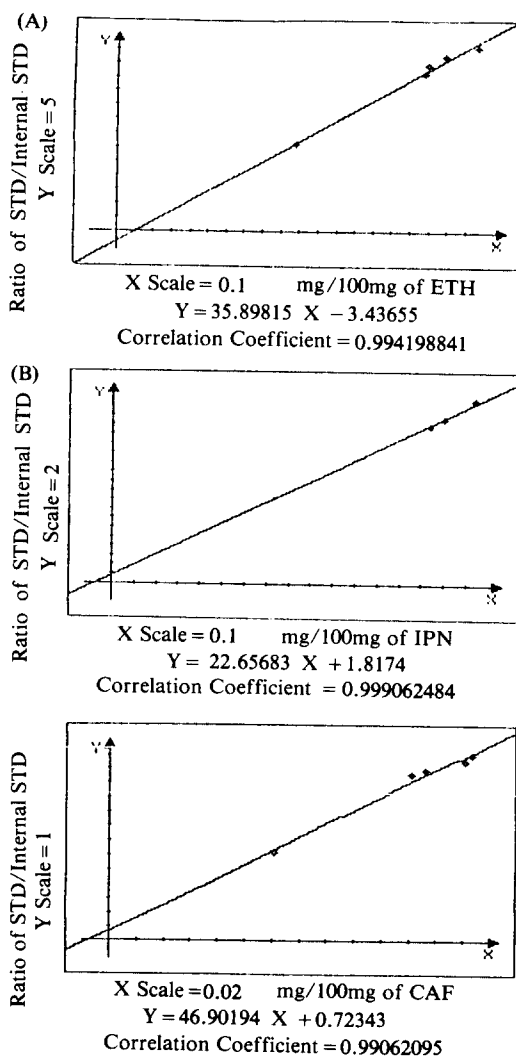


Fig. 4. Calibration curve of ethenzamide (A), isopropylantipyrene (B) and caffeine (C) by GC.

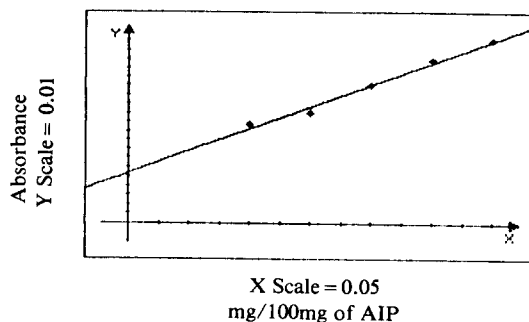


Fig. 5. Calibration curve of allylisopropylacetylurea by UV method.

$Y = 0.244X + 0.546$   
Correlation coefficient = 0.991280396

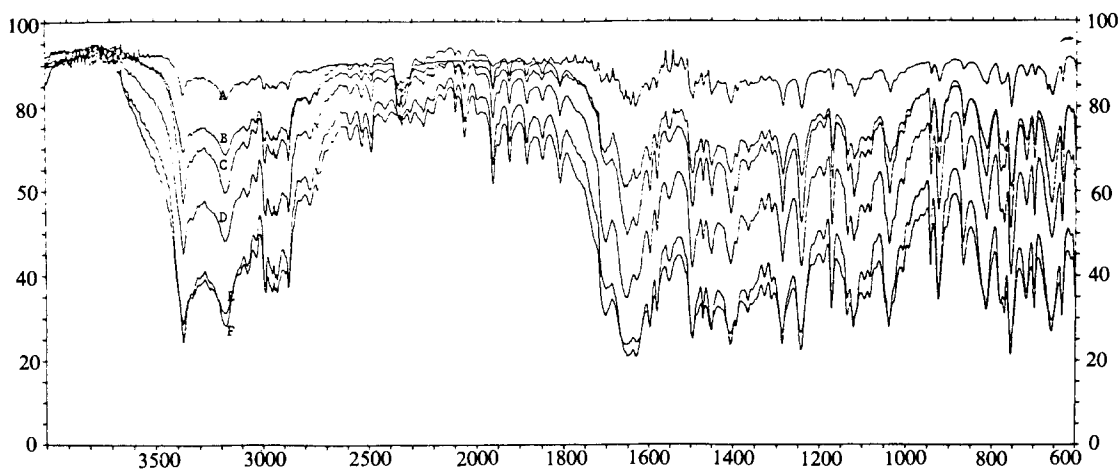


Fig. 6. Spectra of various mixing ratio of sample and dispersing medium (sample: KBr).

(A) 1: 1000 (B) 2: 1000 (C) 10: 1000 (D) 20: 1000 (E) 100: 1000 (F) 200: 1000

aromatic out-of-plane bending. Component IPN has absorption bands at 718 and 611 $\text{cm}^{-1}$ , attributed to the aromatic =CH out-of-plane bending. Component CAF has absorption bands at 1598 $\text{cm}^{-1}$ , attributed to mono substituted amide of -CO-NH-R and 1598 $\text{cm}^{-1}$ , due to -NH bending. Component allylisopropylacetylurea (AIP) has absorption bands at 1244 and 815 $\text{cm}^{-1}$  attributed to aliphatic =CH out-of-plane bending. In this manner, 19 characteristic peaks of four-component were assigned. The wavenumbers of 19 peaks selected are 1598, 1580, 1495, 1405, 1394, 1367, 1330, 1244, 1173, 1122, 1040, 944, 925, 869, 815, 718, 657, 632 $\text{cm}^{-1}$ .

#### Reproducibility of reflectance readings

In quantitative spectrophotometry, the intensities of characteristic absorbance of the compounds in a given system at selected wavelengths are related to the concentrations of the compounds.

Disadvantage of DRS is the lack of reproducibility of band intensity because of variation of the scattering coefficient each time the sample is loaded into the cell. In this study the problem was alleviated more or less by grinding the sample for the same time (10min).

Using improved DRIFT cell and taking precaution to insure a homogeneous sample of relatively uniform particle size, it was possible to get reproducibility of reflectance readings for three different samples of identical concentration (Table V).

#### Acquirement of regression equation

Through predescribed procedure, the ratio of these 19 peaks to reference peak were calculated after selection of the characteristic peaks of four components. Nineteen peaks were selected as re-

ference peaks during process of regression, in turn. Stepwise means insertion of variables in turn until the regression equation is satisfactory.

The nature of this set and the techniques applied increase quality accuracy, although the set already includes various deviations. Possible deviations include molecular interactions, spectrometer nonlinearities, nonlinearities due to dispersion in the index of refraction, or nonzero baselines arising from reflections or absorptions from windows, substrates, or solvents. LSMR yielded estimates of pure component spectra from a set of known standard mixture spectra.

Ultimately, the final regression equations chosen by stepwise procedure of ETH(eq. 1), IPN(eq. 2), CAF(eq. 3) AIP(eq. 4) are presented in Table I, II, III, and IV.

Every regression coefficient obtained was more than 0.9, that is, regression equation obtained by stepwise multiple regression well described the content of each component in sample.

Although LSMR used in this study have been applied to the infrared spectroscopy, they can be applied to any spectroscopy in which the intensity of the spectral features is reproducible and can be estimated as being linearly related to component concentration. An advantage stems from the fact that spectra are obtained as a digital signal on the associated computer. As presented in Table VI, the major advantage of performing spectroscopic analysis of multi-component system is the speed at which an analysis can be performed.

#### Correlation between analytical methods

From these above mentioned regression equations, the contents of 9 unknown samples were cal-

**Table I. Regression equation for ethenzamide****(A) reflectance value itself (B) by K-M equation (C) by inverse K-M equation**

STEPWISEMULTIPLE REGRESSION RESULT for the DATA of FILE, [B:ETH1.16]

FINAL SOLUTION	11:13:57	12-03-1987
(A) Regression Number: 8		
ETHENZAMIDE [REF] = -252.1214		
- 1.002846E+03	[1580/815]	
+ 5.013530E+02	[1450/815]	
+ 3.850906E+02	[1405/815]	
+ 8.310061E+02	[1394/815]	
+ 4.845372E+01	[1330/815]	Eq. 1-a
- 7.900057E+02	[1244/815]	
+ 3.219376E+02	[632/815]	
Coefficient of Determination = 0.9720029		
STD deviation of estimate = 1.855983		

STEPWISEMULTIPLE REGRESSION RESULT for the DATA of FILE, [B:ETH2.08]

FINAL SOLUTION	11:26:22	12-03-1987
(B) Regression Number: 15		
ETHENZAMIDE [K.M] = 43.18238		
+ 1.713269E+02	[1580/1330]	
- 6.480390E+01	[1450/1330]	
- 9.171926E+01	[1405/1330]	
- 6.880346E+01	[1394/1330]	
+ 8.653514E+01	[1244/1330]	Eq. 1-b
- 2.815584E+01	[718/1330]	
Coefficient of Determination = 0.9579268		
STD deviation of estimate = 2.192442		

STEPWISEMULTIPLE REGRESSION RESULT for the DATA of FILE, [B:ETH3.17]

FINAL SOLUTION	11:29:07	12-03-1987
(C) Regression Number: 5		
ETHENZAMIDE [I.K.M] = -7.654908E-02		
+ 3.587903E-02	[1244/718]	
+ 6.764111E-02	[944/718]	
- 9.777924E-02	[925/718]	
+ 1.034026E-01	[632/718]	Eq. 1-c
Coefficient of Determination = 0.8386387		
STD deviation of estimate = 2.118389E-03		

culated. The contents from these equations are shown in Table VII and contents determined by GC and UV are shown in Table VIII and Table IX.

In practice, it is found that the K-M function is

valid only over a limited concentration range as it is shown in CAF and AIP (Table X, Fig. 9-(B), and Fig. 10-(B)). The primary reasons for this appear to be arised from two basic assumptions which are not

**Table II. Regression equation for isopropylantipyrine  
(A) reflectance value itself (B) by K-M equation (C) by inverse K-M equation**

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STEPWISEMULTIPLE REGRESSION RESULT for the DATA of FILE, [B:IPN1.19]

FINAL SOLUTION 11:30:36 12-03-1987

(A) Regression Number: 6

ISO-ANTIPYRINE [REF] = 55.86804

- 5.466804E+02 [1580/632]

+ 2.938229E+02 [1450/632]

+ 5.171860E+02 [1394/632]

- 3.072690E+02 [1244/632]

+ 1.388245E+01 [1122/632]

Eq. 2-a

Coefficient of Determination = 0.9577249

STD deviation of estimate = 1.063813

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STEPWISEMULTIPLE REGRESSION RESULT for the DATA of FILE, [B:IPN2.01]

FINAL SOLUTION 11:32:12 12-03-1987

(B) Regression Number: 10

ISO-ANTIPYRINE [K.M] 6.893046

+ 1.426889E+02 [1580/1598]

- 1.423526E+02 [1405/1598]

- 4.583054E+01 [1394/1598]

+ 1.001167E+02 [1244/1598]

- 3.445352E+01 [718/1598]

Eq. 2-b

Coefficient of Determination = 0.9516309

STD deviation of estimate = 1.137907

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STEPWISEMULTIPLE REGRESSION RESULT for the DATA of FILE, [B:IPN3.16]

FINAL SOLUTION 11:34:56 12-03-1987

(C) Regression Number: 8

ISO-ANTIPYRINE [I.K.M] = 8.349318E-02

+ 1.914769E-01 [1580/815]

- 1.024472E-01 [1450/815]

- 2.537875E-01 [1405/815]

- 8.278175E-02 [1394/815]

+ 2.447529E-01 [1244/815]

- 5.917366E-03 [1122/815]

- 3.581552E-02 [718/815]

Eq. 2-c

Coefficient of Determination = 0.9644009

STD deviation of estimate = 1.533767E-03

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**Table III. Regression equation for caffeine**  
**(A) reflectance value itself (B) by K-M equation (C) by inverse K-M equation**

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STEPWISEMULTIPLE REGRESSION RESULT for the DATA of FILE, [B: CAF1.09]

FINAL SOLUTION 11:43:32 12-03-1987

(A) Regression Number: 8

CAFFEINE [REF] = 170.6882

+ 1.476056E+02 [1580/1244]

- 1.493623E+02 [1405/1244]

- 1.291433E+02 [1394/1244]

- 5.997299E+00 [1330/1244]

- 2.483262E+01 [657/1244]

Eq. 3-a

Coefficient of Determination = 0.9502005

STD deviation of estimate = 0.4614394

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STEPWISEMULTIPLE REGRESSION RESULT for the DATA of FILE, [B: CAF2.01]

FINAL SOLUTION 11:45:13 12-03-1987

(B) Regression Number: 8

CAFFEINE [K.M] = 20.815

- 5.802835E+01 [1580/1598]

+ 5.970219E+01 [1405/1598]

+ 1.431797E+01 [1394/1598]

- 4.249064E+01 [1244/1598]

+ 1.495425E+01 [718/1598]

Eq. 3-b

Coefficient of Determination = 0.9424742

STD deviation of estimate = 0.495945

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STEPWISEMULTIPLE REGRESSION RESULT for the DATA of FILE, [B: CAF3.02]

FINAL SOLUTION 11:47:52 12-03-1987

(C) Regression Number: 10

CAFFEINE [I.K.M] = -0.425598

+ 1.442315E+00 [1405/1580]

+ 2.622725E-01 [1367/1580]

- 1.191682E+00 [1244/1580]

- 4.322738E-01 [815/1580]

+ 4.070335E-01 [632/1580]

Eq. 3-c

Coefficient of Determination = 0.9486003

STD deviation of estimate = 6.982587E-03

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valid. Namely, it is assumed that homogenous scattering layer are involved in which scatter and absorption are proportional to the layer thickness. The K-M equation are then written for these layer. Furthermore, as the medium becomes more strong-

ly absorbing, anomalous dispersion effects should play an increasingly significant role. These effects would also lead to deviations from the ideal K-M behavior, since they are not included in theory. By the way, modified inverse K-M equation relation-

**Table IV. Regression equation for allysopropylacetylurea  
(A) reflectance value itself (B) by K-M equation (C) by inverse K-M equation**

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STEPWISEMULTIPLE REGRESSION RESULT for the DATA of FILE, [B: AIP2.16]

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FINAL SOLUTION

11:39:44

12-03-1987

(A) Regression Number: 8

ALLYLISO-ANTYRINE [K.M] = -12.53871

- 2.578218E+01 [1580/815]

+ 3.507637E+01 [1405/815]

+ 1.609638E+01 [1367/815]

- 2.186944E+01 [1244/815]

+ 1.656086E+01 [632/815]

Eq. 4-a

Coefficient of Determination = 0.9416902

STD deviation of estimate = 0.369896

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STEPWISEMULTIPLE REGRESSION RESULT for the DATA of FILE, [B: AIP1.19]

FINAL SOLUTION

11:36:52

12-03-1987

(B) Regression Number: 8

ALLYLISO-ANTIPYRINE [REF] = -7.457288

- 4.070325E+01 [1598/632]

+ 1.632295E+02 [1580/632]

- 5.722318E+01 [1450/632]

- 1.436168E+02 [1394/632]

+ 9.308102E+01 [1244/632]

Eq. 4-b

Coefficient of Determination = 0.9518082

STD deviation of estimate = 0.3362758

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STEPWISEMULTIPLE REGRESSION RESULT for the DATA of FILE, [B: AIP3.01]

FINAL SOLUTION

11:41:28

12-03-1987

(C) Regression Number: 15

ALLYLISO-ANTYRINE [I.K.M] = 0.6090488

- 5.692532E-01 [1580/1598]

- 3.562892E-01 [1495/1598]

+ 1.621255E+00 [1405/1598]

+ 1.058303E-01 [1367/1598]

- 1.316144E+00 [1244/1598]

+ 1.624437E-01 [925/1598]

- 7.209392E-01 [815/1598]

+ 5.493900E-01 [632/1598]

Eq. 4-c

Coefficient of Determination = 0.9730664

STD deviation of estimate = 6.289892E-03

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**Table V. Reproducibility obtained for different sample of identical concentration of ethenzamide, isopropylantipyrine, caffeine, and allylisopropylacetylurea**

Component	Wavelength ( $\text{cm}^{-1}$ )	Sample			Mean Value	Standard deviation
		Sample 1.	Sample 2.	Sample 3.		
ETH	1450	0.32	0.317	0.312	0.316	0.0033
	1405	0.283	0.283	0.271	0.279	0.0057
	1244	0.246	0.248	0.235	0.243	0.0057
	632	0.371	0.38	0.376	0.376	0.0037
IPN	718	0.422	0.43	0.429	0.427	0.0036
	611	0.546	0.561	0.557	0.555	0.0063
CAF	1598	0.321	0.316	0.31	0.316	0.0045
	1580	0.347	0.345	0.34	0.344	0.0029
AIP	1244	0.246	0.248	0.235	0.243	0.0057
	815	0.381	0.384	0.381	0.382	0.0014

**Table VI. Comparison of time between each analytical method**

Gas Liquid Chromatography (GLC)	
Weighing 9 samples and 5 STD	40 min.
Washing apparatus	20 min.
Preparing sample and STD	24 hr.
Warming up GLC	24-hr.
Acquiring Chromatogram (retention time: 15-20 min) (3 duplicates)	16 hr.
<b>TOTAL</b>	<b>ca. 41 hr</b>
Ultra-violet Spectrometry	
Weighing 9 samples and 5 STD	40 min.
Washing apparatus	20 min.
Extraction time	12 hr.
Preparing sample and STD	24 hr.
Regular experimental time	2 hr.
Acquiring UV spectrum(3duplicates)	1 hr.
<b>TOTAL</b>	<b>ca. 40 hr.</b>
DRIFT	
Weighing 9 samples 19 STD, 30KBr	1 hr.
Acquiring sample and STD spectrum	250 min.
Reading Reflectance	30 min.
Performing Regression	20 min.
Calculation of Result	6 min.
<b>TOTAL</b>	<b>ca. 6 hr. 6 min.</b>

ship can be found which linearizes correlation much better than the K-M theory over a extended concentration range.

Correlation coefficient obtained through performing regression with data manipulated by inverse K-M equation was the most favorable as presented in Table VI. Good agreements with conventional method of analysis using known concentrations are shown in figures 7-(c), 8-(c) 9-(c), and 10-(c).

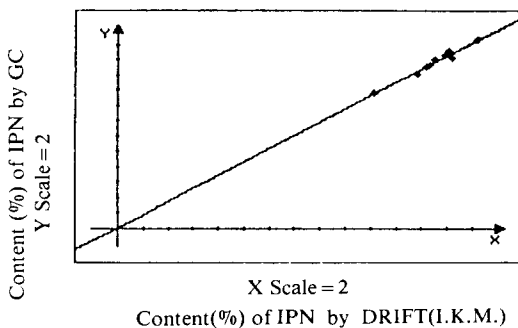
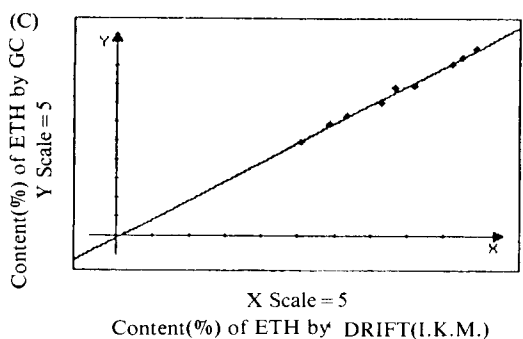
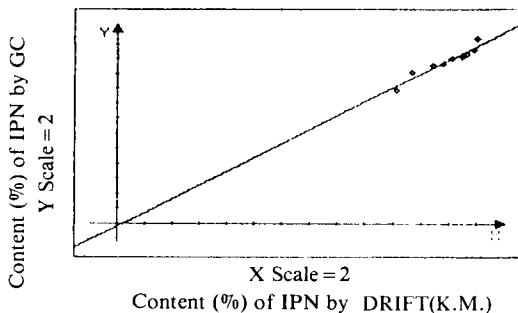
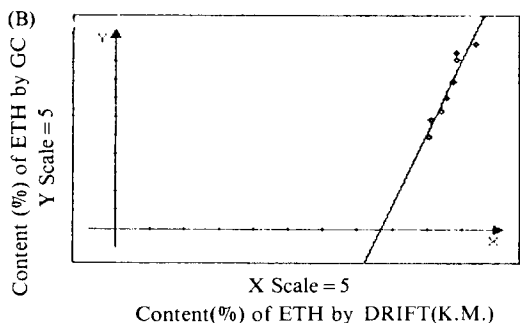
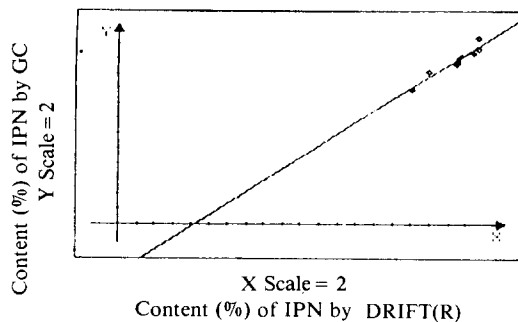
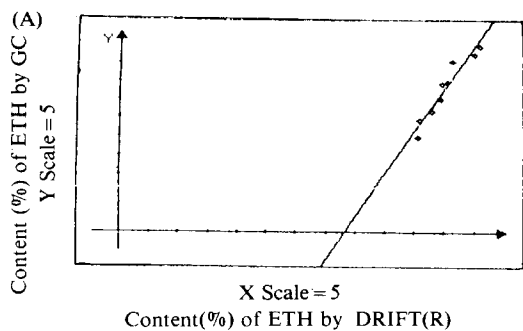
Serious difficulties are encountered in achieving optimal results for more complex systems in which the relationship between concentration and reflectance is nonlinear for one or more compounds, or in which there is mutually interfering reflectance among the components and particularly for systems in which all these occurs simultaneously. Most of these difficulties can be minimized by application of the least-squares procedure to develop equations which best represent the experiment.

Since a set of standard mixture spectra, which cover the expected concentration range of the unknown samples, will include K-M equation deviations due to molecular interactions, spectrometer-related nonlinearities, and dispersion in refractive index, these nonlinear terms were approximated in the analysis as the inverse K-M equation. Therefore, the accuracy of the analysis was dependent on the magnitude of the nonlinearities over the concentration range of the standard mixtures.

DRS could eventually be a major influence in preformulation screening programs and final product quality control.

**Table VII. Content of ethenzamide, isopropylantipyrine, caffeine and allylisopropyl urea in unknown sample calculated from regression equation**

Sample No. of ETH	1	2	3	4	5	6	7	8	9
Content (%)	25.47	29.27	31.84	36.33	38.41	40.83	45.98	47.38	49.36
Sample No. of IPN	1	2	3	4	5	6	7	8	9
Content (%)	18.3	20.22	21.32	21.48	21.88	22.38	22.65	23.28	25.32
Sample No. of CAF	1	2	3	4	5	6	7	8	9
Content (%)	5.51	6.99	7.43	8.28	8.32	8.38	8.35	10.73	10.8-
Sample No. of AIP	1	2	3	4	5	6	7	8	9
Content (%)	3.74	4	4.15	4.7	4.81	4.86	4.93	5.69	5.83

**Fig. 7. Correlation curve of ethenzamide.**

- (A) reflectance value itself  
(B) by K-M equation  
(C) by inverse K-M equation

**Fig. 8. Correlation curve of isopropylantipyrine.**

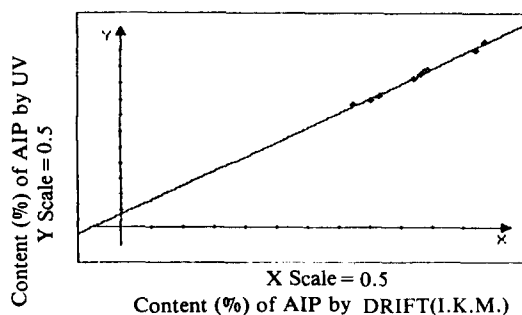
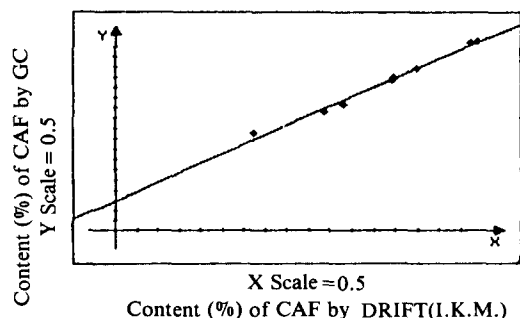
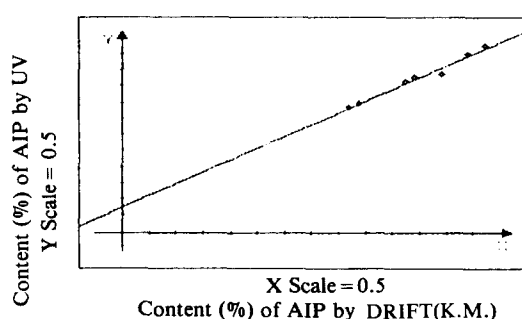
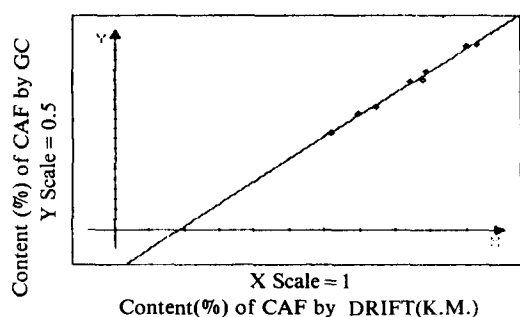
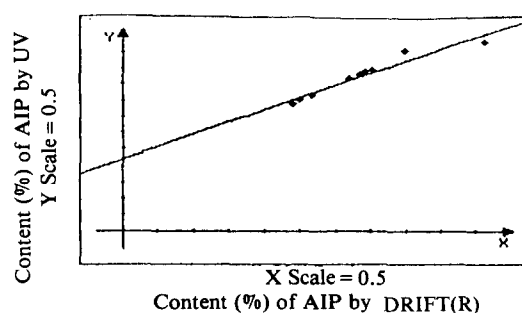
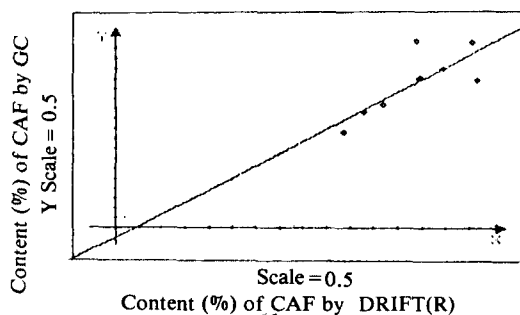
- (A) reflectance value itself  
(B) by K-M equation  
(C) by inverse K-M equation

**Table VIII. Content determined by GC**

Sample No. of ETH	1	2	3	4	5	6	7	8	9
Content (%)	24.65	29.29	31.84	35.25	39.12	36.69	45.28	47.27	49.61
Sample No. of IPN	1	2	3	4	5	6	7	8	9
Content (%)	17.78	20.23	21.22	21.42	22.18	22.38	22.66	23.18	24.78
Sample No. of CAF	1	2	3	4	5	6	7	8	9
Content (%)	4.8	5.78	6.15	7.34	7.44	7.49	7.9	9.21	9.29

**Table IX. Content determined by UV**

Sample No. of AIP	1	2	3	4	5	6	7	8	9
Content (%)	3.86	3.99	4.15	4.66	4.79	4.89	4.92	5.5	5.79

**Fig. 9. Correlation curve of caffeine.**

- (A) reflectance value itself
- (B) by K-M equation
- (C) by inverse K-M equation

**Fig. 10. Correlation curve of allylisopropylacetylurea.**

- (A) reflectance value itself
- (B) by K-M equation
- (C) by inverse K-M equation

**Table X. Relative correlation coefficient between contents determined by GC and UV and contents acquired by DRIFT**

		DRIFT		
		Reflectance corr. coeff.	K-M equation corr. coeff.	Reverse K-M equation corr. coeff.
	ETH	0.961	0.945	0.997
GC	IPN	0.951	0.956	0.989
	CAF	0.778	0.996	0.994
UV	AIP	0.967	0.989	0.996

## CONCLUSION

As presented here, a critical evaluation of the application of reflectance to direct analysis of solid mixture has established the fact that analytically useful data can usually be obtained with samples in powder form. A DRIFT method can afford with reasonable result within considerably short time compared with conventional method, GC and UV. The more samples are quantified, the more time can be saved.

DRIFT spectra over the whole concentration range could be interpreted by means of a inverse K-M equation in which the difference in the scattering coefficient between the sample and the dispersing medium was taken into account. The best quantitative results were obtained in 70-120% concentration range out of contents prescribed in tablet.

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