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原 著

A Study on the Analysis of Isothiazolinone Components by High Performance Liquid Chromatography

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고성능액체크로마토그래피에 의한 Isothiazolinone Components의 분석에 관한 연구

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국 문 초 록

냉각탑, 제지산업, 일반 산업용수 등에 사용되는 산업용 방부제의 유효 성분 isothiazolinone components 즉, 2-methyl-4-isothiazolin-3-one(unchlorinated compound)과 5-chloro-2-methyl-4-isothiazolin-3-one(chlorinated compound)을 고성능액체크로마토그래피로서 분리, 정량하였다. 역상의 C_{18} column (15 cm×3.9 mm I.D.)을 사용하였으며 자외선 검출기의 파장 254 nm에서 methanol-0.4% acetic acid(40 : 60)의 이동상, methanol-0.4% acetic acid(30 : 70)의 추출 및 주입용매 조건으로 HPLC 분리를 시도한 결과 unchlorinated compound는 10~32,400 mg/*l* 범위에서, 그리고 chlorinated compound는 120~107,000 mg/*l*의 범위에서 직선성을 보였다. 내부 표준물질로서 dimethyl phthalate를 사용하였으며, unchlorinated compound, chlorinated compound 및 내부 표준물질의 순서로 분리가 일어났고 총 분리시간은 6.41분이었다. 상기의 조건으로 시험물질을 분석, 정량한 결과 예측농도치에 근접한 수치를 얻었다.

Keywords : Isothiazolinone components, 2-methyl-4-isothiazolin-3-one, 5-chloro-2-methyl-4-isothiazolin-3-one, HPLC

Introduction

Isothiazolinone product is one of new preservatives used in cooling tower, paper mill, and general industrial waters.[1] It is also effective in controlling bacteria and fungi in the manufacture and storage of dispersed pigments, such as kaolin clays, titanium dioxide, calcium carbonate and others.[2] Its broad-spectrum activity, excellent physical and chemical compatibility with anionic, nonionic and cationic surfactants and most organic and inorganic compounds and low toxicity at recommended use levels provide formulators with an effective, economical, and environmentally acceptable alternative to other commercial biocides. It dose not contain or generate formaldehyde and is easy to formulate (1.5% solution is supplied as an aqueous solution), so that it gains advantage over the other preservatives.

The active ingredients of the isothiazolinone product are unchlorinated compound (2-methyl-4-isothiazolin-3-one) and chlorinated one (5-chloro-2-methyl-4-isothiazolin-3-one). Methods preferred for the analysis of preservatives are chromatographic methods, especially high performance liquid chromatograph (HPLC).[3-11] Although several methods were satisfactory in respect to separation[12], no offical method has been published for the isothiazolinone components. This study was performed to search for an alternative method in order to show flexible operating conditions of HPLC and to reduce assay time.

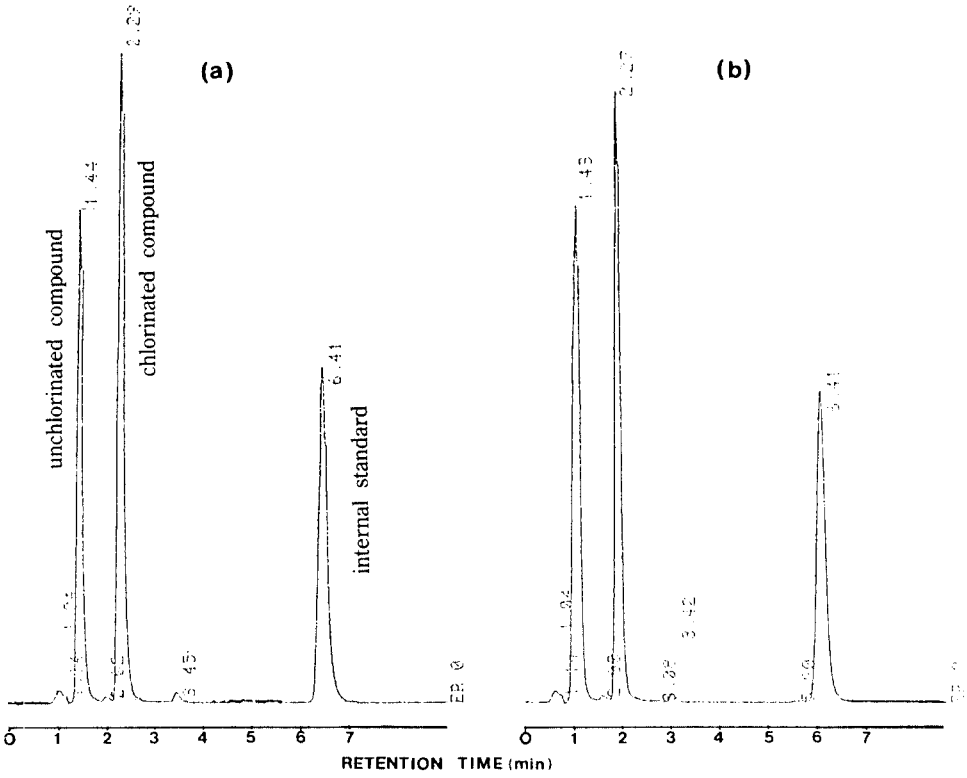

Fig. 1. Chromatogram of isothiazolinone components. Conditions; methanol-0.4% acetic acid (40 : 60) at the flow rate of 1.0 ml/min, detection at UV 254 nm. (a) standard sample (b) test material.

Experimental

1. Reagent, extraction procedure, and sample preparation

Methanol and dimethyl phthalate was HPLC grade and other chemicals used were analytical grade purity. Dimethyl phthalate was used as internal standard.

Internal standard solutions were prepared by dissolving 1.0 ml of dimethyl phthalate in methanol-0.4% acetic acid (30 : 70, v/v), in a 1000 ml volumetric flask. For the preparation of standard solution, 1.0 ml of standard sample and 20.0 ml of internal standard solution were diluted with solvent mixture in a 100 ml volumetric flask and mixed thoroughly. The solution was filtered through a 0.45 μm Millex® -HV filter (Millipore, U.S.A.) adding through a 10 ml syrnge. The filtrate was diluted with injection solvent mixture by the ratio of 1 : 10. Aliquot of the diluted filtrate of standard solution or test material was injected through a fixed loop into the liquid chromatograph system. The mobile phase was a mixture of water, methanol, and acetic acid.

2. HPLC condition

The chromatographic equipment consisted of Waters associates (Milford, MA, U.S.A.) system, M590 pump, delivering a flow rate of 1.0 ml/min, a Rheodyne injector with 100 μl loop, a Nova-Pak C_{18} column (15 cm×3.9 mm I.D.), an M490E UV /VIS detector with 254 nm wavelength, and an M 746 integrator.

Results and Discussion

Optimization of analysis was carried out with standard injections by various condition at room

Table 1. Comparison of assay results of standard sample by HPLC

Assay trial	Extraction solvent[a]	Injection solvent[a]	Mobile phase[a]	Peak shape	Retention time (minutes)
1	55 : 45	30 : 70	30 : 70	Cigar	17.42
2	30 : 70	55 : 45	40 : 60	Flat-top[b]	6.45
3	55 : 45	55 : 45	30 : 70	Cigar	14.07
4	30 : 70	30 : 70	30 : 70	Sharp	14.65
5	30 : 70	30 : 70	40 : 60	Sharp	6.41
6	55 : 45	30 : 70	30 : 70	Sharp	14.51

[a]Methanol-0.4% acetic acid (v/v).
[b]Two isothiazolinone components were overloaded and internal standard was poorly separated.

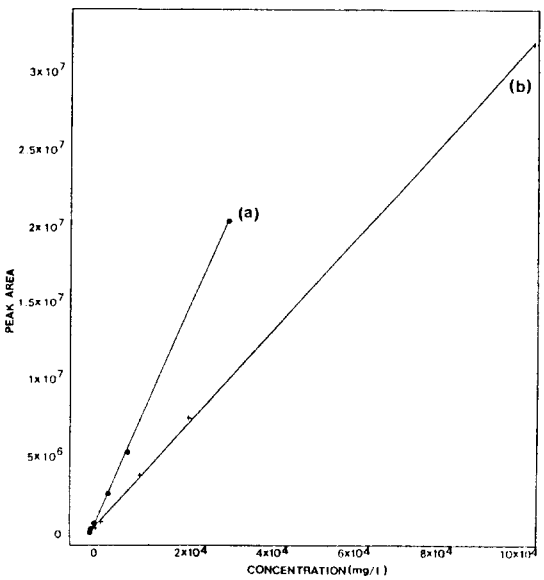

Fig. 2. Calibration curves obtained for standard solution of isothiazolinone components. (a) 2-Methyl-4-isothiazolin-3-one (b) 5-chloro-2-methyl-4-isothiazolin-3-one.

temperature. Table 1 shows the compared assay results obtained with the solvent mixture and mobile phase. A good separation of two isothiazolinone components was achieved with the extraction and injection solvents of methanol-0.4% acetic acid (30 : 70, v/v) and the mobile phase of methanol-0.4% acetic acid (40 : 60, v/v). Elution profiles for a standard solution and test material of the components are shown in Fig. 1.

The two isothiazolinone components and internal standard eluted separated from each other in the order 2-methyl-4-isothiazolin-3-one, 5-chloro-2-methyl-4-isothiazolin-3-one, and internal standard with retention times of 1.44, 2.29, and 6.41 minutes, respectively by the set of solvent mixture and mobile phase in assay trial 6 in Table 1. Each component excellently separated and the total chromatographic run time could be reduced drastically compared with another previous method.[12] In the assay trial 2, the two components were separated as single peak, but internal standard

Table 2. Determination of isothiazolinone components in test material of commercial product

Test material	Content detected (%)			Content expected, total (%)
	Unchlorinated compound	Chlorinated compound	Total	
1	0.734	2.378	3.112	3.5
2	0.715	2.306	3.021	3.5
3	0.753	2.462	3.215	3.5
4	3.744	12.275	16.019	15.0
5	0.460	1.196	1.656	1.5
6	0.443	1.170	1.613	1.5
7	0.772	2.356	3.128	3.5
8	0.920	2.295	3.215	3.5
9	3.586	10.677	14.263	14.0
10	3.716	10.616	14.332	14.0

was not. So the assay conditon of trial 6 could be recommended for the analysis of the two isothiazolinone components.

The method's linearity was checked. Fig. 2 gives calibration plots for the two isothiazolinone components by the above assay condition. The calibration curve shows detector response is linear from 10~32,400 mg/*l* of unchlorinated compound and from 120~107,000 mg/*l* of chlorinated one. The repeatability of the peak areas was also checked. On average it was found that the standard deviation for the peak areas based on 3 runs to be better than 1% when using a 16,000 mg/*l* of the two components in standard sample concentration.

Test materials of commercial products containing the two isothiazolinone components were determined by the above analytical procedure. The contents found agreed well with the expected as shown in Table 2 and the reproducibility of this product was acceptable.

Conclusion

A simple and rapid high-performance liquid chromatographic method was proposed for the separation and determination of two isothiazolinone components, 2-methyl-4-isothiazoline-3-one (unchlorinated compound) and 5-chloro-2-methyl-4-isothiazolin-3-one (chlorinated compound) in a preservative. The separation was achieved on a reversed-phase C_{18} column using methanol-0.4% acetic acid (40 : 60) as the eluent and methanol-0.4% acetic acid (30 : 70) as the extraction and injection solvents. The UV detector responses at 254 nm were linear from 10~32,400 mg/*l* of unchlorinated compound and from 120~107,000 mg/*l* of chlorinated one. Total chromatographic run time could be reduced to 6.41 minutes. The contents of the two components in test material of commercial product by the assay procedure were agreed with the expected concentration.

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