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Coupling of TLC and UV-spectrophotometry for quantification of methyl nicotinate and diethylamine salicylate in ointments

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1. Introduction

Methyl nicotinate, a rubefacient drug, is usually combined with diethylamine salicylate, an analgesic agent with high penetrative value, to relieve the pains of fibrosis, muscular and arthritic rheumatism [1]. Ointments are the most commonly chosen pharmaceutical dosage form for such type of local treatment.

However, a problem concerning the determination of mixtures containing methyl nicotinate and diethylamine in such combination derives from the fact that admixed dispensed additives in the base are interfering factors. Thus, methyl nicotinate and diethylamine salicylate can be quantitatively determined only after suitable chromatographic separation. PFANDL and MAYER [2] described a separation procedure on thin layers of silica gel F_{254} using a mixture of acetonitrile, methanol, and water (45:5:1; by volumes), and recommended a HPLC-system for quantitative determination of methyl nicotinate and diethylamine salicylate in some heparin-containing analgesic gels.

The present work describes a chromatographic system for better separation and identification of methyl nicotinate and diethylamine salicylate in their mixtures with parabens in ointments. UV-Spectrophotometric measurement is successfully applied for determination of the studied drugs in their mixtures found in some of market formulations.

2. Experimental

2.1. Instruments

DU-7 videorecording uv/visible spectrophotometer, Beckman Instruments Inc., CA-U.S.A., was attached to a strip computerized recorder; and, UV-240 Graphicord uv/visible recording spectrophotometer attached to a Graphic Printer PR-1, Shimadzu, Japan. Both instruments were calibrated with matched pairs of 1-cm quartz cells before use.

2.2. Reagents and materials

Chemicals used throughout this investigation were for analytical uses, and a spectrophotometric grade ethanol (Uvasol, E. Merck, Darmstadt-F.R.G.) was used for the spectrophotometric measurements.

Reference sample of methyl nicotinate purchased from Les Etablissements Livaucan Lasirotte, Lyon-France, batch No. 85.031.01, was utilized without previous treatments. The sample was complying with British Pharmacopoeia 1980 requirements [3]; and the purity was 99.15 ± 0.15% as determined according to the B.P. 1980 method [3] in non-aqueous medium; titrating with 0.1 M $HClO_4$ and detecting the end point potentiometrically.

Authentic diethylamine salicylate obtained from Givaudan Corp., New York-U.S.A., batch No. J 14, was used as supplied. The sample was complying with the Martindale Extrapharmacopoeia requirements [1]; and the purity was 98.80 ± 0.20% as determined colorimetrically through the salicylate contents after acidolysis and adding ferric ammonium sulphate and measuring the developed violet red colour at 530 nm [4].

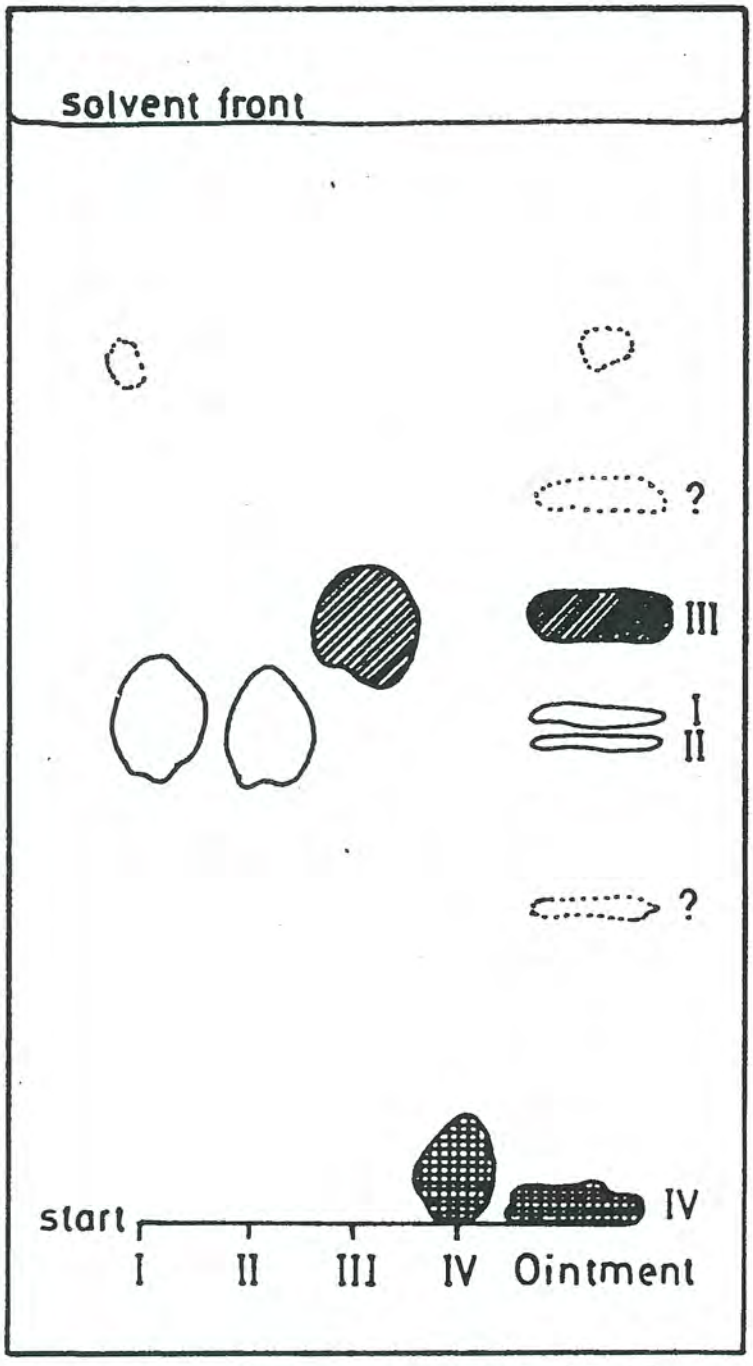
Laboratory prepared thin-layer, 0.25 mm, of silica gel GF_{254}-Type 60 (E. Merck, Darmstadt-F.R.G.) on glass plates, 10 cm × 20 cm and 20 cm × 20 cm, were prepared and activated according to STAHL [5].

Developing system composed of benzene + diethyl ether + methanol + ammonia solution (60:30:8.5:1.5; by volume), it has to be mixed freshly and to be used only once.

2.3. Procedures

2.3.1. TLC-Separation and identification

Weigh accurately about 2 g of the ointment in a small beaker, extract with ethanol, 1 × 10 ml, then 2 × 5 ml, filter into a 25-ml calibrated flask through Whatman No. 1 filter paper, then complete to volume using the same solvent. Apply 10 µl of alcoholic extract of the ointment by aid of a spotting microsyringe as a band onto the starting line. Equal volumes, 10 µl, of



Adsorbent: Silica gel GF 254 (0.25 mm)
Develop. System: C_6H_6 + $(Et)_2O$ + CH_3OH + NH_4OH = 60 : 30 : 8.5 : 1.5 (by vol.)
Visualization: UV (254 nm)

Fig. 1 Typical tl-chromatogram of resolved propyl paraben (I), methyl paraben (II), methyl nicotinate (III), and diethylamine salicylate (IV).

alcoholic solutions of authentic methyl nicotinate (0.40%), diethylamine salicylate (2%), methyl paraben (3.5%), and propyl paraben (3.2%) were applied separately side by side in portions as usual, evaporate the solvent after each addition, then allow to develop in a suitable chromatographic jar containing the developing system. The front of the solvent mixture is allowed to run 12—15 cm above the starting line before removing the plates and allowing the solvent to evaporate in air (fuming cupboard).

All separated bands and spots can be visualized and located by examining the plates under ultraviolet light (254 nm). Another special visualizing spray reagents can be also utilized for some of the isolated substances, such as ferric alum (1%, aqueous) gives violet-brown spots with diethylamine salicylate; König's reagent [6—8] shows yellow coloration, within one hour, with methyl nicotinate; spraying with ammonical cupric sulphate (1%, aqueous) then exposure to carbon disulphide and ammonia vapours yields brown coloured spots, within 10 minutes, with diethylamine radicle of the salicylate salt.

Table 1 represents the R_f-values of methyl nicotinate, diethylamine salicylate, methyl and propyl parabens; while figure 1 shows typical tl-chromatographic separation of such substances.

Table 1 The retention values (R_f) of methyl nicotinate, diethylamine salicylate, methyl paraben, propyl paraben, resolved from their mixture in Baumalgin® ointment.

Pharmaceutical Substance	R_f-Value*
Methyl nicotinate	0.55
Diethylamine salicylate	0.03 (start line)
Methyl paraben	0.45
Propyl paraben	0.46

* TLC-Separation on 0.25 mm thin layers of silica gel GF_{254} on glass plates using a developing system containing benzene, diethylether, methanol, and ammonia solution (conc.) = 60:30:8.5:1.5; by volumes.

2.3.2. UV-Spectrophotometric measurements

Macerate, separately, the under UV-light located and scratched layers containing methyl nicotinate, diethylamine salicylate, and the reference amount of each, in a small beaker using spectroscopic-grade ethanol, and transfer by filtration into 25-ml calibrated flasks, then complete the extracts to volume using the same extracting solvent.

Measure the absorbance of the obtained sample extracts against blank at 264 nm, methyl nicotinate, and 297 nm, diethylamine salicylate, using a 1-cm cell. The concentration can be determined either by referring to the absorbance of resolved reference standard, and/or from the determined $A^{1\%}_{1\,cm}$-values of each of the two active ingredients; table 2 gives the determined $A^{1\%}_{1cm}$-values and the corresponding calculated molar absorptivities of both drugs.

3. Results and discussion

The UV-scanning (400—200 nm) of ethanolic solutions of methyl nicotinate and diethylamine salicylate is shown in figure 2. While diethylamine salicylate shows maximal absorption at 297 nm without interferences from methyl nicotinate, i.e. it can be quantitatively determined at its λ_{max}, methyl nicotinate has its maximal absorption at 264 nm but with minor interference from the diethylamine salicylate. In such a case, VIERORDT's method [9] and modified VIERORDT's method [10, 11] can be applied for the simultaneous spectrophotometric determination of the two components in certain ratios. Ointments usually contain methyl nicotinate and diethylamine salicylate in a ratio of 1 to 5 or even more, where the UV-scanning of mixtures of the two drugs in such a ratio exhibits a typical maximum of the predominating drug, i.e. diethylamine salicylate, at 297 nm, while the absorption of the other appears mostly in a shoulder form. This problem can be

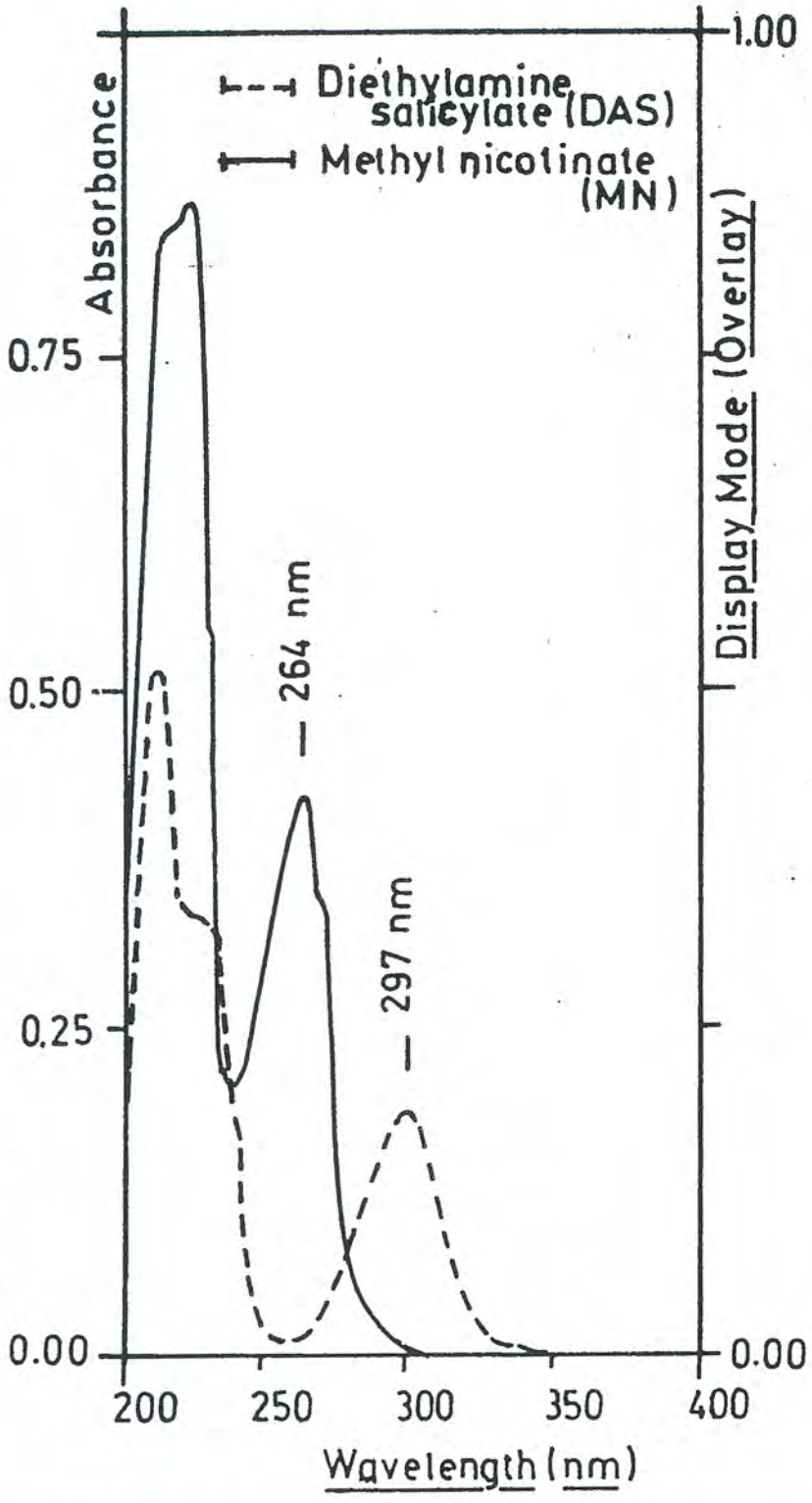


Fig. 2 UV-scanning (400—200 nm) of ethanolic solutions of methyl nicotinate (λ_{max} = 264 nm) and diethylamine salicylate (λ_{max} = 297 nm).

even solved by applying the derivative spectrophotometry [12, 13], but the spectral interferences caused by ointment matrix complicate the spectrophotometric determinations.

An examination of those mixtures requires, first at all, a separation of the components. Thus, they are quantitatively determined after preliminary separation by chromatographic means [2]. TL-Chromatographic separation of methyl nicotinate may include its main degradation product nicotinic acid, diethylamine salicylate, methyl and propyl parabens, can be done on 0.25 mm thin-layers of silica gel GF_{254}-Type 60 utilizing a developing mixture containing benzene, diethyl ether, methanol, and ammonia (60:30:8.5:1.5; by volumes). Diethylamine salicylate, as well as

nicotinic acid if present as main degradation product of methyl nicotinate run very slowly or even remains at the start line, while methyl nicotinate moves faster, and the parabens lay in between; the R_f-values are given in table 1. The adopted chromatographic system gives better resolution than that recommended by PFANDL and MAYER [2] (fig. 1).

UV-Spectrophotometric quantification of the TLC-resolved active compounds can be followed by measuring at 264 nm and 297 nm for methyl nicotinate and diethylamine salicylate. Any nicotinic acid may be present on the start line with diethylamine salicylate can be quantitatively determined at its λ_{max} at about 262 nm. So, the method is a stability-indicating one for methyl nicotinate. The presence of any nicotinic acid with diethylamine salicylate can visualized on the developed chromatogram by exposure to cyanogen bromide and aniline vapours, a special visualizing reagent for pyridine derivatives [6—8]. Methyl nicotinate can be also visualized utilizing the same reagent as it can not differentiate between nicotinic acid, its amide and esters [14].

Table 2 The $A^{1\%}_{1\,cm}$-values and molecular absorptivities (ε) of methyl nicotinate and diethylamine salicylate.

Pharmaceutical Substance	λ_{max} (nm)	$A^{1\%}_{1\,cm}$-value		(ε) ($\times 10^{-3}$)
		determined	literature	
Methyl nicotinate	264	226.3**	230[3,8]	3.103
Diethylamine salicylate	297	181.5**	—	3.835

** Mean of at least six separate determinations

Table 3 Spectrophotometric determination and recovery of added methyl nicotinate and diethylamine salicylate in a market preparation (Baumalgin® ointment*)

Sample Assay			
Labelled (mg · g^{-1})		Found (mg · g^{-1})	
Methyl nicotinate	diethylamine salicylate	Methyl nicotinate	diethylamine salicylate
20	100	18.55	96.38
		18.10	96.62
		18.25	97.50
		17.79	97.01

Control Experiment (Standard Addition)

Standard (Added, mg)		Standard (Found, mg)		Recovery (%)	
Methyl nicotinate	diethylamine salicylate	Methyl nicotinate	diethylamine salicylate	Methyl nicotinate	diethylamine salicylate
10	50	9.81	50.03	98.10	100.05
		9.98	50.45	99.78	100.90
		10.17	50.49	101.74	100.98
		10.03	49.31	100.30	98.62
			Mean (p = 0.05)	99.98 ±1.50	100.14 ±1.10

* Baumalgin® ointment manufactured by Misr Co. for Pharmaceutical Industries, El-Mataria, Cairo — Egypt; batch No. 905115; each 100 g of the ointment are labelled to contain 2 g of methyl nicotinate and 10 g of diethylamine salicylate in a water-washable base.

Table 2 gives the determined $A_{1cm}^{1\%}$-values and the calculated molar absorptivities of methyl nicotinate and diethylamine salicylate. The results obtained from spectrophotometric measurement of each of the active compounds in Baumalgin® ointment are given in table 3. Recovery experiments of added authentic materials of methyl nicotinate and diethylamine salicylate show excellent percent means of 99.98 ± 1.50 and 100.14 ± 1.10, respectively. It is evident that the described TLC-UV-spectrophotometric method can be applied for the analysis of each of methyl nicotinate and diethylamine salicylate when admixed in ointment formulations with parabens. The method is accurate, sensitive, and gives reproducible results with good statistical validity.

Summary: A method using coupling of thin-layer chromatography (TLC) and UV-spectrophotometry for identification and quantification of mixtures of methyl nicotinate and diethylamine salicylate in the presence of the interfering parabens and other additives in ointment formulations is described. Alcoholic extracts of the ointment are chromatographed on thin layers (0.25 mm) of silica gel GF_{254} utilizing a developing mixture containing benzene, diethyl ether, methanol, and ammonia solution (60:30:8.5:1.5; by volumes). The scratched layers containing each active ingredient are extracted completely with ethanol and the absorbances are measured against a blank of the solvent and compared with those of reference amounts of methyl nicotinate at 264 nm and diethylamine salicylate at 297 nm. The method has been evaluated using standard-addition technique; where recoveries of 99.98 ± 1.50% and 100.14 ± 1.10% were obtained for methyl nicotinate and diethylamine salicylate, respectively. Retention values, $A_{1cm}^{1\%}$-values, and molar absorptivities of each of the two drugs are given.

Zusammenfassung: Es wird eine Methode unter Kopplung von Dünnschichtchromatografie und UV-Spektrofotometrie zur Identifizierung und quantitativen Bestimmung von Gemischen von Methylnicotinat und Diethylaminsalicylat in Gegenwart der störenden Parabene und anderer Additive in Salbenformulierungen beschrieben. Alkoholische Extrakte der Salbe werden auf Dünnschichten (0,25 mm) von Silicagel GF 254 unter Verwendung des Laufmittelgemisches Benzen—Diethylether—Methanol—Ammoniaklösung (60:30:8,5:1,5 v/v) chromatografiert. Die abgekratzten Schichten mit den Wirkstoffen werden vollständig mit Ethanol extrahiert und die Absorptionen gegen einen Leeransatz des Solvens gemessen und mit denen von Vergleichsmengen Methylnicotinat bei 264 nm und Diethylaminsalicylat bei 297 nm verglichen. Das Verfahren wurde unter Verwendung einer Standardzusatztechnik bewertet, wobei 99,98 ± 1,50% Methylnicotinat bzw. 100,14 ± 1,10% Diethylaminsalicylat wiedergefunden wurden. Es werden die Retentionswerte, die $A_{1cm}^{1\%}$-Werte und die molaren Absorptivitäten der beiden Arzneistoffe angegeben.

References: [1] Martindale „The Extra Pharmacopoeia", 28th ed., REYNOLDS, J. E. F. (Edit.), The Pharmaceutical Press, London, 1982, p. 250-No. 2633-k and p. 1626-No. 9253-z. — [2] A. PFANDL, H. MAYER: Pharm. Ztg. **128** (1983) 2822. — [3] The British Pharmacopoeia 1980, Her Majesty Stationary Office, London, 1980, p. 286. — [4] S. A. ISMAIEL: Ph. D.-Thesis, Faculty of Pharmacy, Cairo Univ. 1982, p. 86. — [5] E. STAHL, in: Thin-Layer Chromatography, E. STAHL (Edit.), 2nd ed., Springer Verlag, Berlin, 1969, p. 52. — [6] W. KÖNIG: J. prakt. Chem. **69** (1904) 105; **70** (1904) 19. — [7] K. G. KREBS et al.: in: Thin Layer Chromatography, E. STAHL (Edit.), 2nd ed., Springer Verlag, Berlin, 1969, p. 859. — [8] The Pharmaceutical Codex, 11th ed., The Pharmaceutical Press, London, 1979, p. 557. — [9] A. HEILMEYER: in: Spectrophotometry in Medicine, Adam Highler Ltd., London, 1943, p. 7. — [10] A. L. GLENN: J. Pharm. Pharmacol. **7** (1960) 595. — [11] M. J. CHO, M. PERNAROWSKI: J. Pharm. Sci. **59** (1970) 1333. — [12] M. A. KORANY et al.: Anal. Lett. **17(B12)** (1984) 1373. — [13] S. M. HASSAN, A. G. DAVIDSON: J. Pharm. Pharmacol. **36** (1984) 7. — [14] Y. M. DESSOUKY et al.: J. Pharm. Belg. **29** (1974) 43.

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