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# HPLC-QUANTIFICATION OF DIETHYLAMINE SALICYLATE AND METHYL NICOTINATE IN OINTMENTS

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**ABSTRACT**

A simple liquid chromatographic method for simultaneous quantification of diethylamine salicylate and methyl nicotinate in the presence of parabens and some drug degradation products is demonstrated. The LC-separation of the two drug substances was undertaken on a column packed with μ-Bondapack C18 using 1% aqueous acetic acid-acetonitrile (85 + 15) at a flow rate of 1.7 ml/min at ambient temperature with detection at 254 nm. Gradient mixing was adopted after 12 min to enhance the rapid elution of the component propyl paraben and other firmly adsorbed ingredients in the ointment base. Good percent assay and mean recoveries with low deviations were obtained for each added drug substance. The stability indicating characteristic of the investigated procedure shows its advantage over another HPLC-method.

## INTRODUCTION

Therapeutic combinations of the topical analgesic diethylamine salicylate with the vasodilator methyl nicotinate are commonly prescribed in cases of arthretic rheumatism and for the relief of the pains of fibrosis (1-3). Ointments are the most common dosage form suitable for such type of local treatment. Pfandl and Mayer (4) described the utility of liquid chromatography for the analysis of diethylamine salicylate and methyl nicotinate in heparin containing gels after hydrolyzing the heparin contents. The problem concerning the quantification of drug mixtures may arise in most cases from the with-dispensed additives, e.g. emulsifiers, stabilizers and/or preservatives as in case of ointment formulations. The LC-procedure of Pfandl and Mayer failed for many reasons to be adopted for the determination of the two drug substances in admixture with methyl and propyl parabens. Recently, a GLC-procedure for the determination of the two named drugs has been investigated (5). The present work describes a simple HPLC-method for the simultaneous quantification of diethylamine salicylate and methyl nicotinate in the presence of the parabens. The method is also a stability indicating, as the peaks of the degradation products were isolated and defined. The results and

parameters of the investigated method were compared with those of the previously published LC-method.

## EXPERIMENTAL

### Apparatus and Reagents

(a) Liquid chromatograph. Varian Model 5000, equipped with variable-wavelength UV50 detector, Rheodyne Model 7125 valve injector with 20-μl loop, Varian Model 9176 recorder, and controlled by Varian data system (DCS 111L). The column utilized is a stainless steel, 30 cm x 4 mm i.d., packed with 10 μm μ-Bondapack C18 bonded coat, Waters Associates, Milford-Mass. Optimum parameters are as follows: mobile phase, acetonitrile (A) and 1% aqu. acetic acid (B) each was filtered through a suitable Millipore filter and degassed using a Branson 1200 ultrasonic-bath. The separation is performed using 15% of (A) and 85% of (B) at a flow rate of 1.70 $ml.min^{-1}$ for 12 min; then changing (A) to 75% and (B) to 25% at a 2.5 $ml.min^{-1}$ flow rate.

(b) Solvents and Reagents. HPLC-grade $CH_3CN$ (HiPer Solv, BDH, Poole-England), all-glass bidist. water, and gl. acetic acid (BDH, Poole-England), diethylamine salicylate (Givaudan Corp., New York-U.S.A.) BN J14 with 98.80 ± 0.20% purity as determined according to the BP-1988 method (6), methyl nicotinate (Les Etablissements Livaucan Lasirotte,

Lyon-France) BN 85.031.01 with 99.15 ± 0.15% purity as assessed by adopting the BP-1988 method (7). Paracetamol, 10 mg% aqu. solution, is utilized as internal standard.

(c) Standard Solutions and Sample Preparation

Standard series. From the aqueous stock solutions of diethylamine salicylate, 2.5 $mg.ml^{-1}$, or methyl nicotinate, 0.5 $mg.ml^{-1}$, prepare serial dilutions of each drug substance to cover concentration ranges 100-500 $\mu g.ml^{-1}$ for diethylamine salicylate and 20-100 $\mu g.ml^{-1}$ for methyl nicotinate using the mobile phase as solvent. Add the internal standard, 8 $\mu g.ml^{-1}$, to each drug solution before completion to volume. Calculate the standard ratio for triplicate injections of each solution.

Sample preparation. Weigh about 150-250 mg of the ointment in a beaker and transfer quantitatively into a 50-ml flask using about 40 ml of the mobile phase, and add 4 ml of the internal standard solution. Shake for 5 min and complete to volume using the same solvent mixture. After mixing well filter and inject 6 replicates each 20 $\mu l$ onto the column. To obtain the content of each individual drug substance, calculate the sample ratio, or from standard curves of peak area vs concentration of standard solutions. When using the direct sample/standard assay, the following formula can be adopted for calculations;

$C(mg\%) = SP/ST \times C_{std} \times D/W$ ; where :

SP & ST are the sample and standard ratio; i.e. each compared with the internal standard.

D is the dilution, Cstd is standard concentration, & W is the weight of the ointment (g).

Recovery experiment. To an ointment extract containing 20 mg diethylamine salicylate and 4 mg methyl nicotinate, add 10-30 mg and 2-6 mg of the two drug substances, in order, in 25-ml flasks. Add 2 ml of the internal standard solution and complete to volume using the mobile phase. Inject each solution 3 times and calculate the ratio-values, i.e. the areas due to each drug concentration compared with the area of the internal standard.

## RESULTS AND DISCUSSION

Assay trials on the ointment containing diethylamine salicylate in admixture with methyl nicotinate in the presence of methyl and propyl parabens have been undertaken by adopting the LC-procedure described by Pfandl and Mayer, on LiChrosorb RP-$C_{18}$ column utilizing acetonitrile-water (55+45) as the mobile phase (4). The investigations revealed that diethylamine salicylate elutes in the void volume, i.e. with solvent peak; if the extracting solvent is not the mobile phase. Also, any nicotinic

acid, which is a possible degradation product of the nicotinate ester, shows a peak very near to that of diethylamine salicylate. Practically it was found that the examined preparation contained some nicotinic acid that may result from the hydrolysis of the corresponding methyl ester in the presence of moisture. The presence of nicotinic acid with the corresponding methyl ester and other ingredients in the pharmaceutical preparation could be assessed on a thin-layer of silica gel G by using a developing solvent mixture containing cyclohexanemethylethyl-ketone-gl. acetic acid, (10+10+0.3); the presence of the prototype p-hydroxybenzoic acid could also be differentiated from its methyl and propyl esters, i.e. parabens. It was noticed too that the peak due to the methyl paraben, k'-value of 1.06, interferes with that of methyl nicotinate, k'-value of 1.30, especially when the concentration of the ointment in the extract was $\geqslant$ 0.3 g%.

The reversed-phase column was found to be more suitable for the separation of the two main drug substances from each other, and from their degradation products, as well as from the parabens. The LC-procedure of Pfandl & Mayer was modified by using 1% aq. acetic acid instead of water, this allows good separation of nicotinic acid from diethylamine salicylate, but the peak of methyl nicotinate suffers

from pronounced tailing; the tailing factor $\geq$ 3. The use of a $\mu$-Bondapack $C_{18}$ column solved such a problem with complete elution in shorter time. The k'-values for diethylamine salicylate and methyl nicotinate were 4.88 and 1.75, respectively, at a flow rate of 1.70 $ml.min^{-1}$. Fig 1a demonstrates the LC-resolution of diethylamine salicylate and methyl nicotinate compared with the internal standard. The major component drugs in the ointment as well as the methyl paraben were eluted completely after about 11 min; but the propyl paraben and some other minor ingredients in the base took about 40 min to elute. Resortion to the gradient elution, by decreasing the polarity of the mobile phase and increasing the flow rate to 2.5 $ml.min^{-1}$ after the elution of the two major drug components (12 min), shortened the overall analysis time to 18 min Propyl paraben showed a k'-value of 9.63, and two other peaks, one due to the identified nicotinic acid and the other suspected to represent p-hydroxy benzoic acid gave k'-values of 0.21 and 7.40, respectively. To minimize possible errors, paracetamol was introduced as an internal standard. The chosen substance separates quite reasonable, k' values = 0.73, from the two examined drugs and possible degradation products. The peak symmetry was assessed by measuring its tailing factor; it was found to be 1.33 and 1.31 for diethylamine salicylate and methyl nicotinate, respectively.

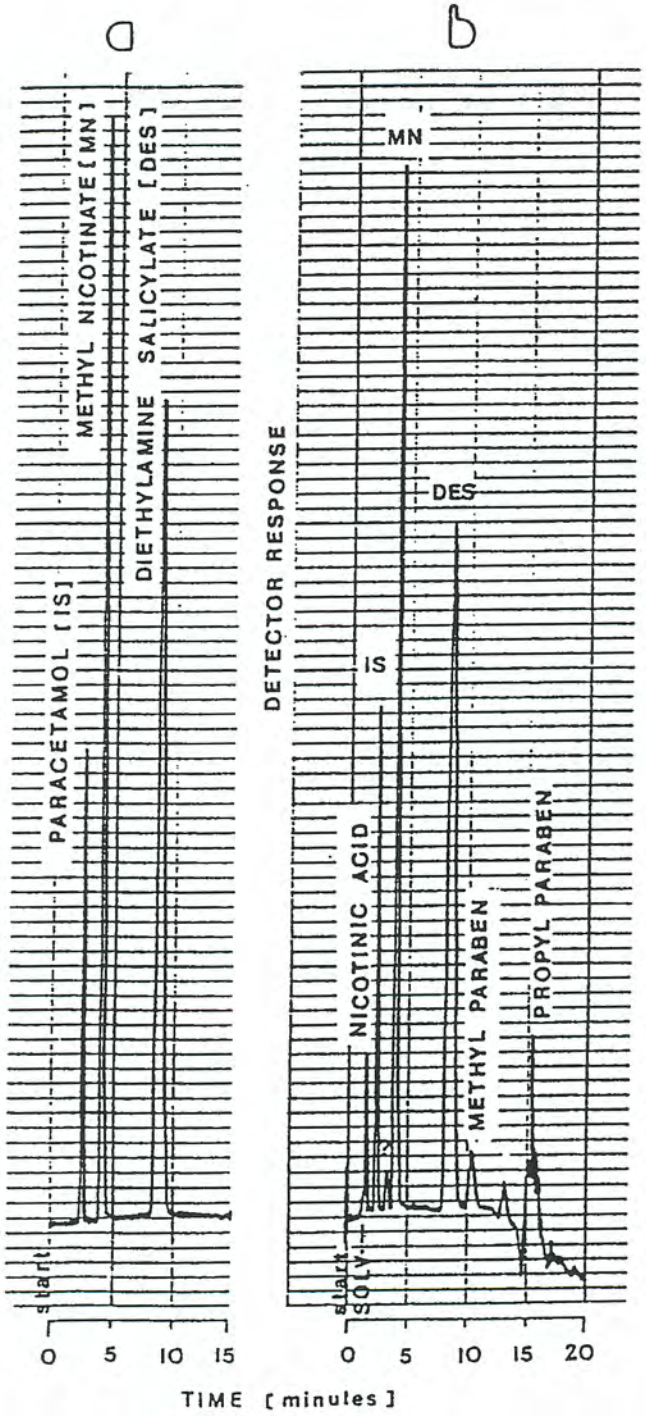


FIGURE 1. The LChromatogram of (a). resolved reference diethylamine salicylate, methyl nicotinate & paracetamol as an internal standard; and (b). eluted drug substances and the internal standard in addition to the present other with dispensed ingredients in the studied ointment.

TABLE 1

Assay of diethylamine salicylate and methyl nicotinate in ointment*

| Ointment weight (g/100 ml) | Claimed contents (mg%) (DES) | (MN) | n | Found (Average for n experiments) Diethylamine Salicylate (DES) std. ratio | sample ratio | content (mg%) | Assay (%) | Methyl Nicotinate (MN) std. ratio | sample ratio | Content (mg%) | Assay (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 0.494 | 49.40 | 9.88 | 4 | 5.959 | 5.920 | 49.75 | 100.75 | 3.632 | 3.198 | 8.75 | 88.52 (95.06)+ |
| 0.506 | 50.60 | 10.12 | 4 | 5.959 | 6.037 | 50.74 | 100.28 | 3.632 | 3.175 | 0.77 | 86.77 (93.02) |
| 0.293 | 29.34 | 5.87 | 3 | 3.657 | 3.640 | 29.95 | 101.93 | 2.172 | 1.920 | 5.28 | 90.01 (95.65) |
| 0.207 | 20.70 | 4.14 | 3 | 2.418 | 2.488 | 20.61 | 99.58 | 1.310 | 1.310 | 3.60 | 86.90 (95.32) |
| | | | | x = 100.60<br>n = 14<br>SD = 1.21 | | | | x = 87.88 (94.66)<br>n = 14<br>SD = 1.78 (1.46) | | | |
| | | | | Y = m + bc (mg%); m : 0.0196<br>b : 0.119<br>r : 0.9995 | | | | 0.009<br>0.362<br>0.99998 | | | |

*Baumalgine® ointment, product of Misr Co. for Pharm. Ind., S.A.A., El-Mataria, Cairo-ET., each 1 g is labelled to contain 100 mg diethylamine salicylate and 20 mg methyl nicotinate in a water-washable base.

+Total nicotinate contents; i.e. methyl nicotinate plus the free nicotinic acid in the samples.

The relative $R_t$ for diethylamine salicylate and methyl nicotinate were 6.69 and 2.40, in order, based on that of paracetamol. Fig. 1b shows the LC-separation of the drug components in the investigated ointment. Application of the proposed method gives consistent percent mean contents for diethylamine salicylate, 100.60 ± 1.21 (n = 14), and methy nicotinate, 87.88 ± 1.78 (n = 14). The low assay results in case of the

TABLE 2

Recovery and reproducibility of diethylamine salicylate and methyl nicotinate

| | Diethylamine Salicylate (DES) | | | Methyl Nicotinate (MN) | | |
|---|---|---|---|---|---|---|
| | Amount (mg%) | | | Amount (mg%) | | |
| | Dispensed | Added | % (n) | Dispensed | added | % (n) |
| Recovery | 20.7 | 10 | 99.14 (3) | 4.14 | 2 | 100.14 (3) |
| | | 20 | 99.58 (3) | | 4 | 100.61 (3) |
| | | 30 | 101.62 (3) | | 6 | 99.50 (3) |
| | | x | 100.29 | | | 100.09 |
| | | SD | 1.10 | | | 1.19 |
| | | n | 9 | | | 9 |
| Reproducibility | (50 mg%); | | 99.52 | (10 mg%); | | 100.22 |
| | | | 99.68 | | | 100.77 |
| | | | 100.26 | | | 99.94 |
| | | | 99.65 | | | 100.50 |
| | | | 99.06 | | | 99.44 |
| | | | 101.83 | | | 98.57 |
| | | x | 100.00 | | | 99.99 |
| | | SD | 0.98 | | | 0.77 |
| | | n | 6 | | | 6 |

nicotinate ester can be attributed mainly to its ease ability to hydrolyse giving the prototype nicotinic acid, which can be resolulted and quantified simultaneously with the intact drug substance. Total nicotinate contents, i.e. the methyl ester plus the free acid, are some what higher for the same analyzed samples, 94.66 ± 1.46 (n = 14). Table 1 collects the obtained results from the analysis of the Baumalgine®

ointment. The accuracy, checked by the recovery testing of added varying amounts, 50-150% of the claimed amounts of each drug substance, as well as the reproducibility of the results, show sufficient consistent accuracy, table 2. The published LC-method of Pfandl & Mayer (4) gave higher assay results for diethylamine salicylate, 124.03 ± 1.62 (n = 5), because of the interference of the solvent peak as well as that due to the present nicotinic acid. Higher results have been also obtained for methyl nicotinate, 95.81 ± 2.50 (n = 5) as a result to the present interferent methyl paraben. The stability indicating characteristic of the investigated procedure shows its advantage over the other LC-method in the accurate quantification of diethylamine salicylate and methyl nicotinate in the presence of their degradation products and some interferents such as the parabens.

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