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# Determination of meprobamate in pharmaceutical dosage forms also containing carbromal by liquid chromatography and indirect photometric detection*

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**Abstract**: In a pharmaceutical form also containing carbromal, meprobamate could not be quantified selectively by classical methods described in pharmacopoeias due to a significant interference from carbromal. Consequently, reversed-phase HPLC methods have been developed to separate the two active ingredients using indirect photometric detection to visualize and determine meprobamate which has very poor chromophoric properties. Different parameters influencing the sensitivity of the indirect response, such as the nature of the highly absorbing compound added to the mobile phase (the marker) as well as the methanol content and the pH of this phase, have been studied. Two chromatographic systems containing benzoic acid or cinnamic acid as the marker, have been optimized and validated. Good linearity and reproducibility have been obtained with both systems but the cinnamic acid method has the advantage that meprobamate and carbromal can be determined simultaneously at 273 nm.

**Keywords**: *HPLC; indirect photometric detection; meprobamate; carbromal; pharmaceutical dosage forms.*

## Introduction

The quantitative analysis of meprobamate in pharmaceutical dosage forms also containing carbromal cannot be performed by the classical methods described in pharmacopoeias [1–2]. Indeed, these methods are based on the hydrolysis of meprobamate and subsequent titration of ammonia after distillation [1] or on colorimetry [2] and give rise to a significant interference from carbromal.

High-performance liquid chromatography (HPLC) seems to be an appropriate method for the determination of meprobamate in the presence of carbromal. However, meprobamate has no significant absorbance above 210 nm and direct UV detection at lower wavelengths is not so reliable due to a high baseline noise. Moreover, the choice of organic modifiers which can be added to the mobile phase under these conditions is very limited. On the other hand, several methods for the determination of meprobamate by gas chromatography have been reported but these techniques most often involve complicated sample preparation procedures, including solvent extraction, hydrolysis and derivatization [3–7].

An interesting alternative for the determination of meprobamate by HPLC is the use of indirect photometric detection [8–14]. This technique is based on the addition to the mobile phase of a highly absorbing compound: the marker. The marker is distributed to a certain extent in the stationary phase. When equilibrium is reached, the mobile phase gives a constant and rather high background absorbance signal. On sample injection, the distribution of the marker is disturbed because of mutual interaction effects with sample components and this gives rise to changes of the marker concentration in the sample zones. Deviations from the constant background signal are then detected and appear on the chromatogram as positive or negative peaks. The capacity ratios ($k'$) of these peaks correspond to those of sample components and their areas are proportional to the amount of the components in the sample.

Beside sample peaks, a second kind of peak is usually observed. The latter, called system peak, has constant retention and is characteristic of the chromatographic system itself. In principle, as many system peaks as there are mobile phase components can be generated but most often the chromatographic conditions

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are chosen so that only one retained system peak, which can be related to the marker, is obtained [8–10].

It has been recently shown that the direction and the magnitude of the sample peaks can be predicted by means of simple equations [11–13]. The latter clearly indicate that in reversed-phase HPLC systems, one of the main parameters for the optimization of sensitivity in indirect detection is the retention of the analyte relative to that of the system peak originating from the marker. Guidelines for the design of such chromatographic systems have been described previously [14].

The aim of this paper is to develop and validate HPLC methods using indirect photometric detection for the determination of meprobamate in pharmaceutical dosage forms also containing carbromal. Two different compounds, benzoic acid and cinnamic acid, have been selected as markers and in both chromatographic systems, the composition of the mobile phase has been optimized with respect to detection sensitivity.

## Experimental

*Apparatus*

The chromatographic equipment consisted of an Altex model 110 A pump (Berkeley, CA, USA), and an Altex model 210 injector equipped with a 50 μl loop. The solutes were monitored with a Pye–Unicam model PU 4020 variable wavelength UV absorbance detector (Cambridge, UK) equipped with a 8 μl cell.

A Heto model 02PT 923 waterbath (Birkeröd, Denmark) was used for thermostatting the chromatographic system. The chromatograms were recorded on a Kipp en Zonen BD 9 two channel recorder (Delft, The Netherlands).

The pH of mobile phase buffers was adjusted by means of a model 632 pH meter from Metrohm (Herisau, Switzerland).

*Chemicals and reagents*

Cinnamic acid, benzoic acid and sodium hydroxide were obtained from Merck (Darmstadt, Germany). Phosphoric acid was provided by UCB (Leuven, Belgium). Methanol of HPLC grade was obtained from Janssen Chimica (Geel, Belgium). Meprobamate was purchased from Sigma Chemical Company (St Louis, MO, USA).

All reagents were of analytical grade except cinnamic acid which was a 'for synthesis' product (minimum 98%) and they were used without further purification. Water was of Milli-Q quality (Millipore Corporation, Bedford, MA, USA).

*Chromatographic systems*

The analytical column was a LichroCART cartridge (125 × 4 mm) prepacked with 5 μm Lichrospher RP-18 from Merck.

The compounds were separated in the isocratic mode. In the benzoic acid method the mobile phase consisted of $10^{-3}$ M benzoic acid in a mixture of 0.05 M phosphoric acid (pH 1.9) and methanol (70:30, v/v).

In the cinnamic acid method, the mobile phase consisted of $4.9 \times 10^{-5}$ M cinnamic acid in a mixture of phosphate buffer adjusted to pH 4.8 with sodium hydroxide and methanol (60:40, v/v).

The mobile phases were degassed for 15 min in an ultrasonic bath before use.

*Chromatographic technique*

The flow rate was 0.9 ml $min^{-1}$ and UV detection was performed at 273 nm. The chromatographic column and the connecting tubes preceding the column were thermostatted at 35 ± 0.1°C. Equilibrium was usually reached after passage of 20–40 ml of eluent according to the chromatographic conditions. Peak areas were determined by triangulation.

*Standard solutions*

A stock solution was prepared by dissolving meprobamate and carbromal in mobile phase in order to obtain a concentration of 10 mg $ml^{-1}$ for each compound. The stock solution was sonicated for a few minutes to facilitate dissolution of meprobamate.

The standard solutions were prepared by dilution of the stock solution in mobile phase to reach the following concentration ranges: 0.5–8 mg $ml^{-1}$ in the benzoic acid method and 0.25–4 mg $ml^{-1}$ in the cinnamic method.

*Sample preparation*

Ten tablets containing meprobamate and carbromal as active ingredients were weighed and finely powdered. A portion of the powder, equivalent to about 100 mg of meprobamate, was weighed accurately, transferred to a 50 ml volumetric flask and dissolved in mobile phase. The solution was then diluted with mobile

phase to volume, sonicated for 10 min and finally filtered.

## Results and Discussion

*Selection of the marker*

In order to obtain maximum sensitivity in indirect photometric detection, the compound to be added to the mobile phase (the marker) should have not only high absorptivity but also retention characteristics similar to those of the non-absorbing analyte. Indeed, it has been shown previously [8–14] that the magnitude of the indirect response in reversed-phase systems depends very much upon the retention of the analyte relative to that of the system peak originating from the marker and that a maximum response is obtained when the two peaks are situated very close to each other.

With an uncharged compound such as meprobamate, the sensitivity of the indirect detection is usually somewhat lower than with ionic analytes whether the marker is ionized or not [8, 10, 13]. However, it is advantageous to use ionizable markers in this case since the capacity ratio of the corresponding system peak can be modified by altering the pH of the mobile phase while the retention of meprobamate remains constant.

Among the different compounds tested as markers, two carboxylic acids, benzoic and cinnamic acid, were found to be particularly suitable for the indirect detection of meprobamate. Both compounds have high UV absorbance, the indirect response being monitored at 273 nm where their spectra present a maximum. At pH around 2, both acids are present in the mobile phase in uncharged form; under these conditions, benzoic acid gives rise to a system peak which elutes in the vicinity of the meprobamate peak while the system peak given by the more hydrophobic cinnamic acid is more strongly retained. For the latter compound, buffers of higher pH have been used in the mobile phase in order to decrease the retention of the corresponding system peak.

*Influence of methanol concentration*

As mentioned above, benzoic acid should preferably be in unionized form when it is used as a marker for the indirect detection of meprobamate. In this instance, the capacity ratios of meprobamate and the system peak related to benzoic acid can be modified by addition of methanol to the mobile phase.

The influence of methanol concentration is illustrated in Fig. 1. The capacity ratios of both peaks are affected differently, the retention of meprobamate decreasing more rapidly with increasing methanol concentration than that of the system peak. As shown in Fig. 1, this change in the relative retention gives rise to a reversal of the elution order of the two peaks at a methanol concentration of about 37% (v/v). With benzoic acid as the marker, the relative retention of meprobamate and the system peak — and consequently the sensitivity of indirect detection — can thus be regulated systematically by changing the methanol content of the mobile phase.

The reversal of the elution order is accompanied by a change in the direction of the peaks, in accordance with the usual response pattern observed with uncharged analytes [8, 10, 13]. At a methanol concentration of 45% (v/v), meprobamate, which elutes before the system peak, appears as a positive peak. Under these conditions, however, the two peaks have very low retention and are not completely resolved. At a methanol concentration of 30% (v/v), meprobamate is more retained than the system peak and appears as a negative peak, as shown in Fig. 2. In this case, the peaks are well separated but close enough to give adequate detection sensitivity.

This methanol concentration has been selected for the determination of meprobamate when benzoic acid is used as the marker, although carbromal is too strongly retained under these conditions to be analysed simultaneously.

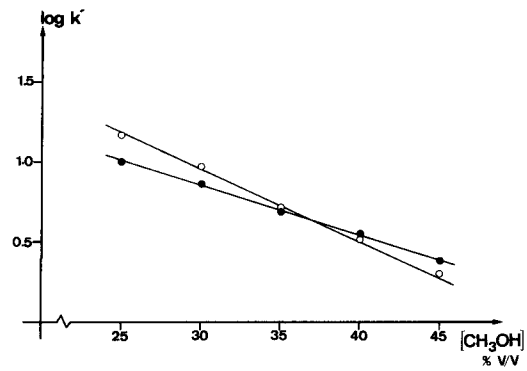


**Figure 1**
Influence of methanol concentration on the retention of meprobamate and the system peak. Mobile phase: $10^{-3}$ M benzoic acid in 0.05 M phosphoric acid–methanol (pH 1.9). Other chromatographic conditions as described in Experimental. ●, System peak (benzoic acid); ○, meprobamate.

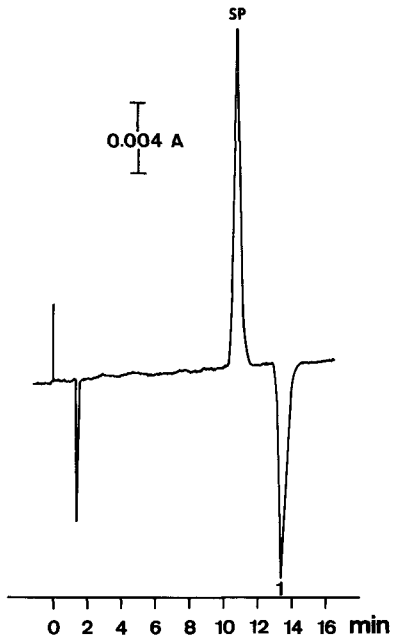


**Figure 2**
Indirect photometric detection of meprobamate with benzoic acid as marker. Mobile phase: $10^{-3}$ M benzoic acid in 0.05 M phosphoric acid–methanol (70:30, v/v; pH 1.9). Other chromatographic conditions as described in Experimental. Solute 1, meprobamate (25 μg). SP, system peak (benzoic acid).

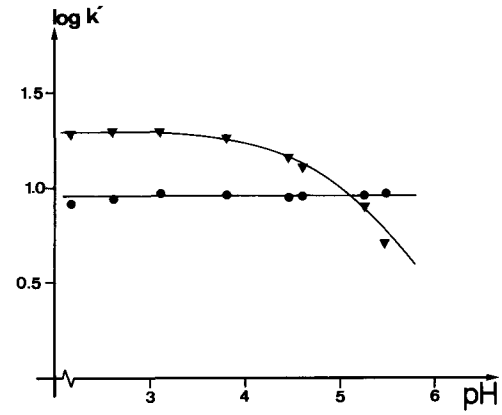


**Figure 3**
Influence of pH on the retention of meprobamate and the system peak. Mobile phase: $4.9 \times 10^{-5}$ M cinnamic acid in phosphate buffer–methanol (70:30, v/v). Other chromatographic conditions as described in Experimental. ▼, System peak (cinnamic acid); ●, meprobamate.

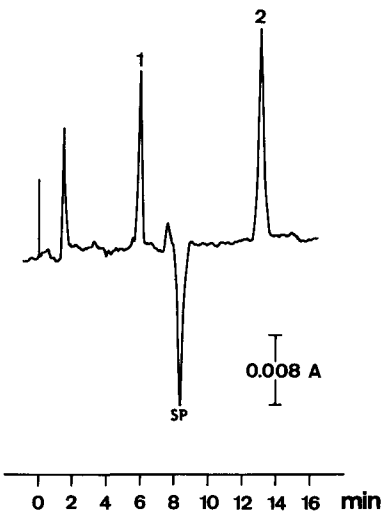


**Figure 4**
Indirect photometric detection of meprobamate with cinnamic acid as marker. Mobile phase: $4.9 \times 10^{-5}$ M cinnamic acid in phosphate buffer pH 4.8–methanol (60:40, v/v). Other chromatographic conditions as described in Experimental. Solute: 1, meprobamate (25 μg); 2, carbromal (16.7 μg). SP, system peak (cinnamic acid).

*Influence of pH*

With cinnamic acid, which has a higher hydrophobic character than benzoic acid, the pH of the mobile phase has been changed in order to obtain a system peak with suitable retention. Figure 3 shows the decrease in capacity ratio of the system peak related to cinnamic acid with increasing pH. A reversal of the elution order of the peaks is observed at pH >5, since the retention of meprobamate is unaffected by pH changes.

A pH value of 4.8 was found to be the most appropriate with respect to sensitivity and peak resolution for the indirect detection of meprobamate with cinnamic acid as the marker. Under these conditions, meprobamate is eluted first and appears thus on the chromatogram as a positive peak, as can be seen in Fig. 4.

In this case, a somewhat higher concentration of methanol can be used (40%, v/v), which permits the simultaneous determination of meprobamate and carbromal (cf. Fig. 4). Although it is more strongly retained than the system peak, carbromal also gives a positive peak, as the negative indirect response is overcompensated by the direct response, the absorptivity of this compound at 273 nm being far from negligible.

*Validation of both methods*

The equations derived from meprobamate and carbromal from linear regression analysis made by plotting the peak area ($Y$) in $mm^2$ versus the analyte concentration ($X$) in mg $ml^{-1}$ are given in Table 1.

The somewhat higher sensitivity in the indirect photometric detection of meprobamate

**Table 1**
Linearity

| | Benzoic acid method | Cinnamic acid method |
|---|---|---|
| Meprobamate ($n = 5$) | $Y = 7.7X - 0.4$ $r^2 = 0.9984$ | $Y = 28.9X + 0.5$ $r^2 = 0.9996$ |
| Carbromal ($n = 5$) | — — | $Y = 96.8X + 4.7$ $r^2 = 0.9998$ |

Detection wavelength: 273 nm (AUFS: 0.16).
Concentration range: 0.5–8 mg $ml^{-1}$ (benzoic acid method); 0.25–4 mg $ml^{-1}$ (cinnamic acid method).

**Table 2**
Reproducibility

| | Benzoic acid method | Cinnamic acid method |
|---|---|---|
| Within-day (RSD %; 1 mg $ml^{-1}$) | | |
| Meprobamate | 0.8 ($n = 5$) | 2.2 ($n = 8$) |
| Carbromal | — | 1.6 ($n = 8$) |
| Between-day (RSD %; 1 mg $ml^{-1}$) | | |
| Meprobamate | 1.9 ($n = 5$) | 3.1 ($n = 5$) |
| Carbromal | — | 2.6 ($n = 5$) |

with cinnamic acid as the marker is related to the higher molar absorptivity of this compound.

The determination coefficients ($r^2$) of the regression lines obtained for meprobamate with both markers (cf. Table 1) indicate that the relationship between the indirect response and the analyte concentration is linear in the concentration range studied.

The precision of the methods was estimated by repeated injections of standard solutions containing 1 mg $ml^{-1}$ of meprobamate and carbromal. The results obtained for within-day and between-day reproducibilities are presented in Table 2. These results are comparable to those obtained with direct detection methods provided the temperature of the chromatographic system is carefully controlled. With cinnamic acid as the marker, however, the ruggedness of the method might be slightly lower than with benzoic acid, since slight pH changes can modify the retention of the corresponding system peak and consequently detection sensitivity.

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