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GC–MS determination of phenobarbitone entrapped in poly-ϵ-caprolactone nanocapsules

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Abstract: In order to evaluate the concentration of a hydrophilic drug, phenobarbitone, in a suspension of poly-ϵ-caprolactone nanocapsules, a gas chromatographic–mass spectrometric procedure, performed after methylation of the drug, was developed and validated. Free phenobarbitone (in solution in the liquid phase), released phenobarbitone (after opening the nanocapsules with ethyl acetate) and total entrapped phenobarbitone (after extraction with methylene chloride), were measured. Experimental results for four lots with various concentrations showed that the highest preparation of entrapped drug (80%) was obtained for a total concentration of 3.64 mg ml^{-1} in the nanocapsule suspension.

Keywords: *GC–MS determination; phenobarbitone; nanocapsules; poly-ϵ-caprolactone.*

Introduction

Nanoparticles are colloidal particles which have been widely studied as drug delivery systems [1]. These colloidal drug carriers can enhance the efficacy and reduce the toxicity of drugs [2]. Nanocapsules are particles with a diameter of 10^{-7} m and a shell-like wall in which the active principles are either encapsulated or on which the drug(s) are adsorbed or attached [3].

These new drug carriers are used in the area of drug targeting, because they can modify the distribution of drugs. They can also play a role in the pharmacokinetics; absorption, elimination and metabolism, of drugs [4, 5]. Biodegradable lipid polymers can also be used in order to encapsulate compounds which are otherwise too hydrophilic to pass through gastrointestinal barriers [6].

Phenobarbitone is an antiepileptic drug which is still used worldwide [7] and its metabolism is well known [8]. In order to study the influence of nanoencapsulation on the biodegradation of this hydrophilic drug, nanocapsules containing this barbiturate were prepared. Phenobarbitone was dissolved in an oily solvent, benzyl benzoate, and encapsulated into a poly-ϵ-caprolactone polymer [6], according to the procedure described in Fig. 1 [9].

It was then necessary to develop an analytical method in order to evaluate the concentration of the drug in the nanocapsules suspension. Usually the total drug present in a nanocapsules suspension is measured; less frequently, the free drug is distinguised from the entrapped drug [10]. For this analytical purpose, the separation of the nanocapsules from the aqueous supernatant is then generally performed by ultracentrifugation and seldom by ultrafiltration [10]. The determination of phenobarbitone can be performed by gas chromatography both directly without derivatization [11] and after methylation [12].

This paper describes a method for the determination of phenobarbitone in a nanocapsules suspension. The method comprised a gas chromatographic–mass spectrometric procedure (GC–MS), performed after methylation of phenobarbitone. The method was validated [13].

Experimental

Apparatus

A Hewlett–Packard 5890 gas chromatograph (Palo Alto, CA, USA) equipped with a splitless capillary inlet system was used. A Hewlett–Packard fused-silica capillary column (25 m × 0.22 mm i.d., 0.11-μm film thickness)

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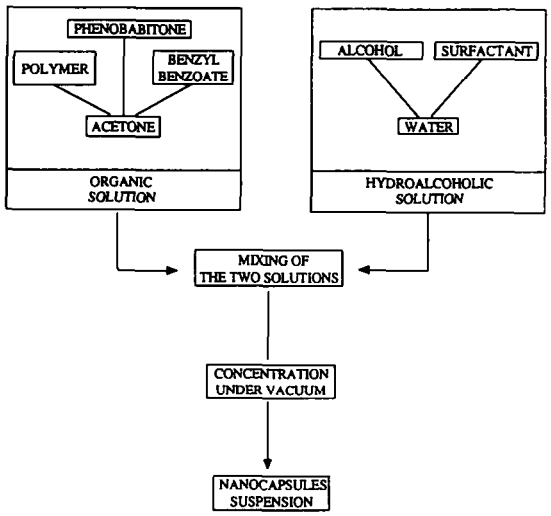

Figure 1
Encapsulation process.

coated with cross-linked 5% phenylmethylsilicone was used. The carrier gas was helium at an inlet pressure of 62 kPa.

A Hewlett–Packard 7673A liquid autosampler, operated in the fast mode for splitless injection, was used in conjunction with the gas chromatograph. Before each injection, the 10-μl injection syringe was automatically rinsed out first with six 10-μl volumes of methanol and then with three 10-μl volumes of the sample solution. Then 3 μl of the sample solution was injected.

A Hewlett–Packard 5970A MSD mass spectrometer, operated in the electron-impact mode, was directly interfaced with the 5890 gas chromatograph by the capillary column and was used in the selected-ion monitoring mode (SIM).

Ultracentrifugation was performed with a Beckman L8-55 ultracentrifuge (Beckman Instruments, Berkeley, CA, USA), equipped with a 40TR rotor.

Chromatographic conditions

The oven temperature was maintained at 120°C for 1 min, increased at 10°C min^{-1} to 270°C, and then held at 270°C for 3 min. The injector and transfer line temperatures were maintained at 240°C.

In the full-scan mode, the scan range was selected from *m/z* 50–400. The mass spectra of barbitone (barbital) and phenobarbitone after methylation (Fig. 2) showed that the following ions should be monitored in the SIM mode: *m/z* 184 and 169 for barbitone, corresponding to $(M-C_2H_4)^{+\cdot}$ = 184 and $(M-C_2H_4-CH_3)^{+}$ = 169; *m/z* 260 and 232 for phenobar-

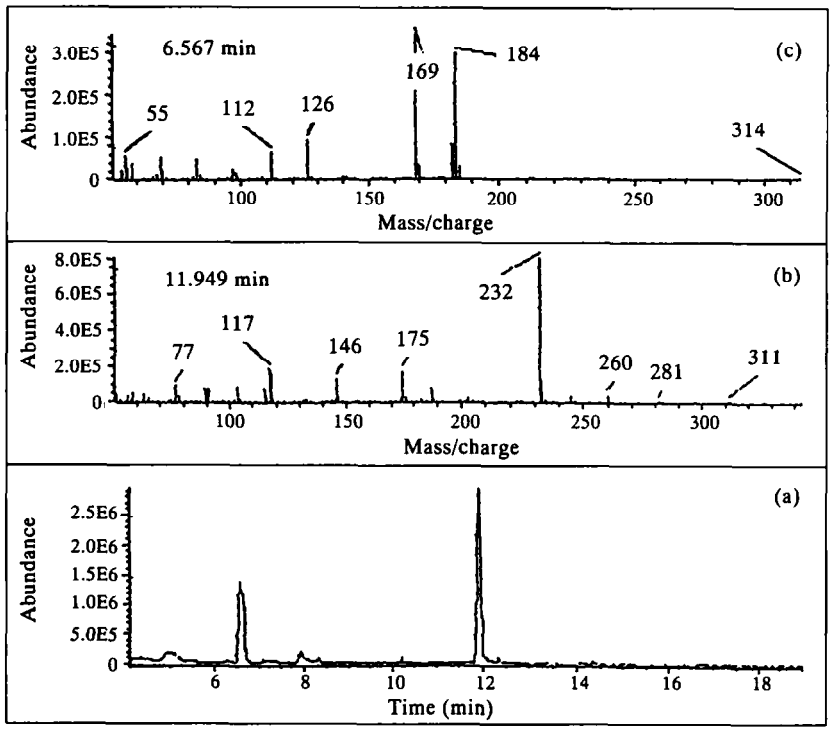

Figure 2
Chromatogram of a solution of phenobarbitone (PB) and barbitone (VE) after methylation (a). Mass spectra of methylated PB (b) and methylated VE (c).

bitone, corresponding to $(M)^{+\cdot} = 260$ and $(M-C_2H_4)^{+\cdot} = 232$.

Reagents and chemicals

Phenobarbitone (PB) (Rhône-Poulenc-Rorer, Paris, France); barbitone (VE) (Serva, Heidelberg, Germany); trimethylanilinium hydroxide (TMAH) 0.2 M in methanol (Methelut^R) (Supelco, Bellefonte, PA, USA); methanol (Carlo Erba, Milan, Italy); methylene chloride (Prolabo, Paris, France); and ethyl acetate (Aldrich, Milwaukee, WI, USA) were used. All the solvents were of HPLC grade.

Internal standard and standard curves

The stock solution of the barbitone [internal standard (IS)] in ethyl acetate (1 mg ml^{-1}) was prepared weekly and stored at 4°C. Solutions of phenobarbitone in ethyl acetate (2 mg ml^{-1}) were prepared each day before use. Working solutions with ethyl acetate were prepared in order to obtain concentrations of 1.0, 0.8, 0.6, 0.4, 0.2 and 0.1 mg ml^{-1} for phenobarbitone, and of 0.25 mg ml^{-1} for the IS, for the construction of standard curves.

Sample preparation

Process 1. Evaluation of PB in the aqueous phase and in the nanocapsules. Ten millilitres of the nanocapsules suspension were centrifuged at 50,000*g* for 45 min in order to obtain a clear supernatant liquid which was then removed by aspiration.

Evaluation in the aqueous phase.

The liquid phase was extracted twice with methylene chloride (50 ml). The organic phases were collected and dried using anhydrous sodium sulphate and then evaporated under reduced pressure. The residue was dissolved in 20 ml of methylene chloride. To 1 ml of this solution were added the IS solution (0.5 ml) and ethyl acetate (0.5 ml). The solution was then evaporated to dryness and the residue was dissolved in Methelut^R (100 μl). An aliquot of this solution (1–3 μl) was injected into the GC–MS system.

Evaluation in the nanocapsules.

The sediment was treated according to either method A or method B.

Method A: the sediment was dissolved in ethyl acetate (20 ml). To 1 ml of this solution were added the IS solution (0.5 ml) and ethyl acetate (0.5 ml). One hundred microlitres of this solution were evaporated to dryness and the residue was dissolved in Methelut^R (100 μl). An aliquot of the solution (1–3 μl) was injected into the GC–MS system.

Method B: the sediment was extracted three times with 50 ml of methylene chloride. The organic phases were collected, dried using anhydrous sodium sulphate and evaporated under reduced pressure. The residue was dissolved in methylene chloride (20 ml). To 1 ml of this solution were added the IS solution (0.5 ml) and ethyl acetate (0.5 ml). One hundred microlitres of this solution were evaporated to dryness and the residue was dissolved in 100 μl of Methelut^R. An aliquot of the solution (1–3 μl) was injected into the GC–MS system.

Process 2. Evaluation of PB in the nanocapsules suspension. Ten millilitres of the suspension of nanocapsules were extracted four times with methylene chloride (50 ml). The organic phases were collected, dried using anhydrous sodium sulphate and evaporated to dryness under reduced pressure. The residue was dissolved in methylene chloride (20 ml). To 1 ml of this solution were added the IS solution (0.5 ml). One hundred microlitres of this solution were evaporated to dryness and the residue was dissolved in Methelut^R (100 μl). An aliquot of the solution (1–3 μl) was injected into the GC–MS system.

Table 1
Validation of the method

Theoretical conc. mg ml^{-1}	Found conc.	Intra-assay variability RSD (%)	Accuracy (%)	Inter-assay variability RSD (%)
0.1	0.0871	4.0	87.1	6.7
0.2	0.1830	2.7	91.5	4.8
0.4	0.3992	2.1	99.8	4.7
0.6	0.5501	4.5	91.7	6.6
0.8	0.8487	4.9	106.1	5.1
1.0	0.9663	2.3	96.6	3.6

Table 2
Determination of phenobarbitone in nanocapsules suspensions

		Lot A	Lot B	Lot C	Lot D
Process 1: aqueous phase	Conc. (mg ml^{-1})	0.78	0.79	1.12	0.77
Free PB (F)	Precision (%)	(RSD: 7.3)	(RSD: 9.1)	(RSD: 2.1)	(RSD: 5.7)
	Conc. (%)	62.3	35.6	43.9	21.2
Process 1: nanocapsules, method B	Conc. (mg ml^{-1})	0.47	1.43	1.52	2.87
Total entrapped PB (E)	Precision (%)	(RSD: 6.0)	(RSD: 10.2)	(RSD: 4.0)	(RSD: 3.9)
	Conc. (%)	37.6	64.4	56.1	78.8
Process 1: nanocapsules, method A	Conc. (mg ml^{-1})	0.40	1.25	1.16	2.17
Released PB (R)	Precision (%)	(RSD: 4.7)	(RSD: 6.5)	(RSD: 4.1)	(RSD: 4.9)
	Conc. (%)	31.8	56.4	42.7	59.8
Strongly adsorbed PB	Conc. (mg ml^{-1})	0.07	0.18	0.36	0.70
(A = E − R)	Conc. (%)	5.9	8.0	13.4	19.0
Total (T = F + E)	Conc. (mg ml^{-1})	1.25	2.22	2.64	3.64

Validation

Linearity studied in the SIM mode was observed for concentrations of 1.0–0.1 mg ml^{-1}. The straight-line equation was $y = 0.291\ x + 0.377$ (y = response ratio, PB/VE; x = concentration ratio PB/VE). The correlation coefficient, r, was 0.994. The validation results established for five injections per concentration, and six concentrations, are listed in Table 1.

For the samples of nanocapsules suspension, the intra-assay precision was established for three injections per determination on four lots (A, B, C, D) and the values are listed in Table 2 (RSD %).

Another lot of nanocapsules suspension was prepared according to Fig. 1 in order to obtain a concentration of 4.00 mg ml^{-1}. For this lot, four successive determinations allowed calculation of the inter-assay precision (RSD %). The results were as follows:

Process 1, in the aqueous phase:
1.11 mg ml^{-1} (RSD = 9.6%)

Process 1, method A:
2.10 mg ml^{-1} (RSD = 4.9%)

Process 1, method B:
2.71 mg ml^{-1} (RSD = 4.9%)

Process 2:
3.91 mg ml^{-1} (RSD = 5.5%)

These validation results showed that the directly measured concentration of PB of 3.91 ± 0.21 mg ml^{-1} (Process 2) was consistent with the theoretical values of 4 mg ml^{-1}.

Results and Discussion

Determinations by Process 1, were performed on four lots of nanocapsules suspension, at various concentrations of about 1 mg ml^{-1} (lot A), 2 mg ml^{-1} (lot B), 3 mg ml^{-1} (lot C) and 4 mg ml^{-1} (lot D). The experimental results listed in Table 2 are given both in mg ml^{-1} and in terms of the percentage of the total amount of PB.

In order to check the consistency of the values of total PB calculated from the determination of total entrapped and free PB (T = F + E), two parts of the same lot D were treated according to both Processes 1 and 2 (Table 3), and the values were compared.

The amount of phenobarbitone is partly dissolved in the aqueous phase, partly adsorbed on the polymer and partly dissolved in the encapsulated benzylbenzoate (Fig. 3). The various analytical processes allowed various kinds of phenobarbitone (Fig. 4) to be distinguished. Free PB (i.e. PB dissolved in the aqueous phase) was determined by Process 1 in the aqueous phase. PB released under the action of ethylacetate (i.e. PB dissolved in the encapsulated benzylbenzoate + PB which is adsorbed, but not strongly), was determined by Process 1, method A, in the nanocapsules. Total entrapped PB (i.e. PB dissolved in the encapsulated benzylbenzoate + total adsorbed PB), was determined by Process 1, method B, in the nanocapsules. Strongly adsorbed PB was determined by the difference between total entrapped and released PB. The total amount of PB present in the whole nanocapsules suspension was determined by Process 2.

Conclusions

The results presented in Table 3 show that the sum of partial concentrations of phenobarbitone (T = F + E) is identical to the total concentration of phenobarbitone directly measured.

These results indicate the effectiveness of the analytical technique which is presented here for the determination of phenobarbitone entrapped in nanocapsules; the method may be

Table 3
Calculated and measured total phenobarbitone

Free PB	Conc. (mg ml^{-1})	0.71
(F)	Precision (%)	(RSD: 4.8)
Total entrapped PB	Conc. (mg ml^{-1})	2.87
(E)	Precision (%)	(RSD: 4.0)
Calculated total concentration (T = F + E)	Conc. (mg ml^{-1})	3.58
Directly measured total concentration	Conc. (mg ml^{-1})	3.44
	Precision (%)	(RSD: 6.9)

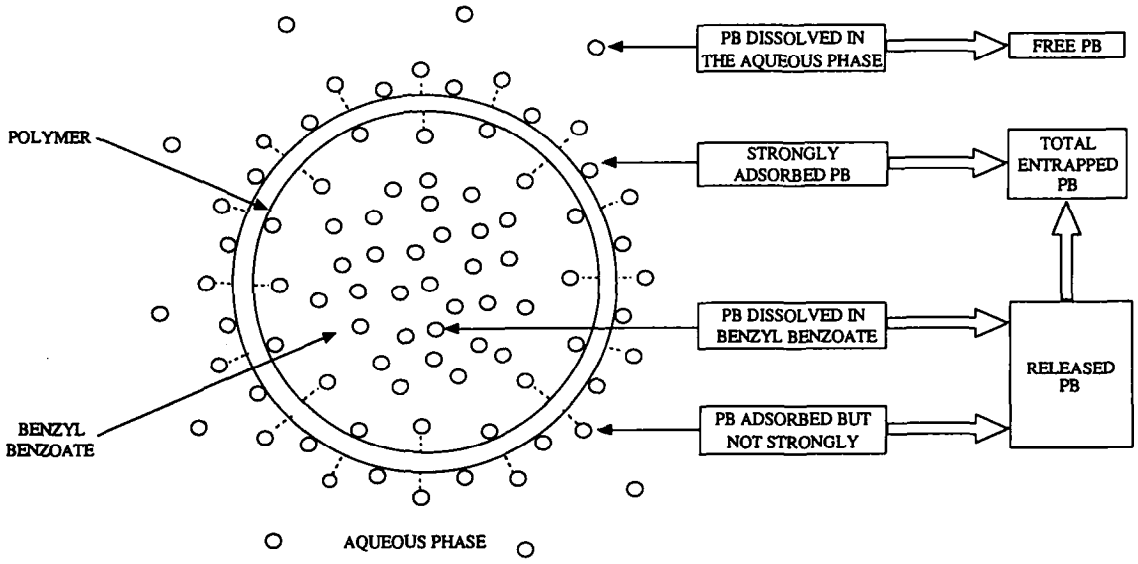

Figure 3
Various kinds of phenobarbitone.

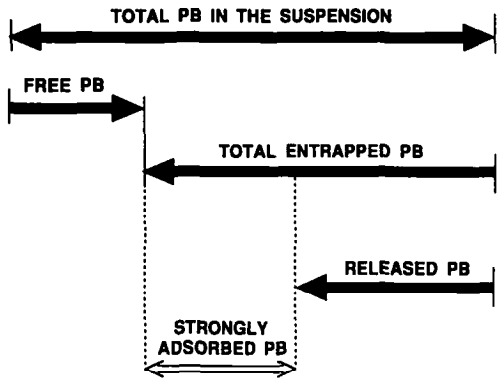

Figure 4
Scheme of phenobarbitone (PB) determination in nanocapsules suspensions.

used to control the quality of the nanocapsules suspension before administration to animals.

From a pharmaceutical point of view, the data displayed in Table 2 show that the total entrapped phenobarbitone increases to nearly 80% of the total amount, when this total concentration reaches 3.64 mg ml^{-1} (lot D) and these results show that further studies must avoid theoretical concentrations lower than 4 mg ml^{-1}.

The particular interest of this analytical technique is not only in allowing the measurement of degree of encapsulation for phenobarbitone, but also in distinguishing between the different ways of trapping the drug, either by simple inclusion inside the nanocapsules or by adsorption to the polymer itself.

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