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Short Communication

Separation and simultaneous determination of methyl paraben and propyl paraben in Choletec® (kit for the preparation of Tc-99m mebrofenin) by high-performance liquid chromatography

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Introduction

Choletec® (kit for the preparation of technetium Tc-99m mebrofenin) [1] is a Tc-99m labelled radiopharmaceutical intended for diagnostic imaging of the hepatobiliary system [2]. The non-radioactive lyophilized product is formulated to contain all the necessary ingredients to synthesize the radiolabelled Tc-99m mebrofenin complex upon addition of the radioisotope Tc-99m in the form of sodium Tc-99m pertechnetate. Each Choletec reaction vial contains 45 mg mebrofenin (2,2′-[(2-[3-bromo-2,4,6-trimethylphenyl)-amino]-2-oxo ethyl)imino] bisacetic acid) as the active, 0.73 mg stannous fluoride as a reducing agent for the sodium pertechnetate, 4.5 mg methyl paraben (methyl 4-hydroxybenzoate) and 0.5 mg propyl paraben (propyl 4-hydroxybenzoate) as preservatives. The structures of mebrofenin, methyl and propyl paraben are shown in Fig. 1.

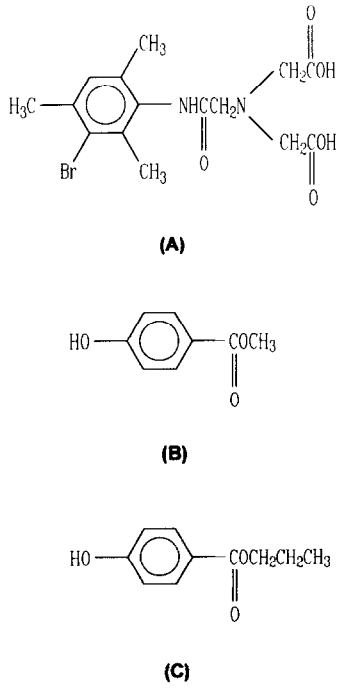

Figure 1
Chemical structure of (A) mebrofenin, (B) methyl paraben and (C) propyl paraben.

Many existing analytical procedures are available for the determinations of methyl paraben and propyl paraben in pharmaceuticals and cosmetics including colorimetry [3, 4], fluorimetry [5], gas chromatography [6, 7], ion exchange chromatography [8], spectrophotometry [9, 10], thin layer chromatography [11, 12] and high-performance liquid chromatography (HPLC) [13–15]. For many methods the sample preparation procedures can be time consuming and may lead to incomplete recovery of the analytes. To overcome such problems, an assay method for the simultaneous determination of methyl and propyl paraben in

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Choletec utilizing LC was developed which is both direct and rapid (<5 min). The method minimized interferences attributed to the major ingredient, mebrofenin, by monitoring at a UV wavelength at which its absorbance contribution is relatively minor and thus eliminating the need for extraction of the two analytes. This study reports on the validation of the method including linearity of response, accuracy, intra-assay precision, limits of detection, ruggedness and system suitability.

Experimental

Chemicals and reagents

Choletec kits and a mebrofenin standard were obtained from Squibb Diagnostics (Princeton, NJ, USA) while USP reference standards were used for methyl paraben and propyl paraben. High purity methanol and acetonitrile and Type I, 18 megohm-cm, organic-free water was used to prepare the mobile phase. Phosphate buffer (0.05 M) was freshly prepared with potassium dihydrogen phosphate (HPLC grade, Fisher Scientific, Springfield, NJ, USA), adjusted to pH 6.8 with 10 M NaOH and filtered through a 0.2-μm Nylon-66 membrane (Alltech Associates, Inc., Deerfield, IL, USA). Individual mobile phase solvents were sparged with helium prior to mixing the mobile phase in the ratio of phosphate buffer–acetonitrile–methanol (35:30:35, v/v/v).

Instrumentation and conditions

The LC system consisted of a SP8700XR ternary solvent delivery system, SP4270 computing integrator, a LABNET data collection system, and a SP8750 organizer module (Spectra-Physics, San Jose, CA, USA) with a Rheodyne 7125 (Cotati, CA, USA) 20-μl nominal volume fixed loop injection valve. The separation was carried out with a 250 × 4.6 mm, 5-μm ODS–Hypersil® column (Shandon Scientific, Ltd). Isocratic elution of this column with the mobile phase delivered at a flow rate of 1.5 ml min^{-1} resulted in a typical operating pressure of approximately 2000 psi at ambient temperature. The absorbance of the mobile phase was monitored at 256 nm at a sensitivity of 0.2 AUFS (1 s time constant) with a Spectroflow 757 (Kratos Analytical, Ramsey, NJ, USA) variable wavelength UV–vis detector. The UV absorption spectra for solutions of methyl paraben, propyl paraben and mebrofenin (10 μg ml^{-1} mobile phase) were determined between 210 and 320 nm with a U-3300 spectrophotometer (Hitachi, Danbury, CT, USA) using 1-cm matched quartz cells versus the mobile phase.

Sample preparation

All lyophilized vials of Choletec were reconstituted with 1.0 ml of 0.9% NaCl and vortexed briefly until the contents were dissolved. An aliquot was removed and diluted 1:600 with the HPLC mobile phase.

Preparation of combination standard solutions

A combination methyl–propyl stock solution was prepared by weighing 110.23 mg of methyl paraben and 12.15 mg of propyl paraben into a 100-ml volumetric flask and dissolving in LC mobile phase. Aliquots of the solution were taken and further diluted with mobile phase to give five working standard solutions with the following concentrations of methyl and propyl parabens, respectively, 8.82:0.972, 6.61:0.729, 4.41:0.486, 2.20:0.243 and 0.882:0.097 μg ml^{-1}. Each of the five combination standard solutions was analysed in duplicate according to the reported HPLC assay procedure.

Preparation of Choletec placebo solution

A Choletec placebo solution, containing only mebrofenin and stannous fluoride, was prepared freshly by adding 4.13 g of 2 M NaOH to a 50-ml volumetric flask containing 30 ml of nitrogen purged water. The solution was warmed to 50°C and to it was added 2.25 g of mebrofenin and 85 μl of a stannous fluoride solution (430 mg ml^{-1} in concentrated HCl). The solution was maintained at 50°C and mixed until all solids were dissolved. Upon cooling, the solution was adjusted to pH 5.4 with 2 M NaOH, diluted to 50 ml with water and filtered through a 0.2-μm Nylon-66 membrane. For LC studies, an aliquot was further diluted 1:600 with HPLC mobile phase.

Propyl paraben stock solution

A stock solution was prepared by weighing 60.51 mg of propyl paraben into a 5-ml volumetric flask. The propyl paraben was dissolved in methanol–water (50:50, v/v) at 50°C, cooled to room temperature and diluted to volume with methanol–water.

Spiked Choletec placebo solutions

Between 27 and 50 mg of methyl paraben

were accurately weighed into each of four, 10-ml volumetric flasks. The methyl paraben was dissolved in approximately 250 μl of methanol. To each flask was added about 8 ml of warm Choletec placebo and an aliquot of the propyl paraben stock solution. The solutions were maintained at 50°C and mixed until the parabens were dissolved. The solutions were cooled to room temperature and diluted to volume with Choletec placebo. Each of the four spiked solutions were further diluted 1:600 with LC mobile phase and assayed in duplicate according to the reported procedure.

Intra-assay precision

Ten replicate, simultaneous methyl paraben and propyl paraben analyses were peformed on a single vial of Choletec for the determination of individual standard deviation and RSD values.

Limit of detection

The combined limit of detection for both methyl and propyl parabens was determined by serial dilution of a reconstituted Choletec kit. Peak response was monitored to a signal-to-noise ratio equal to 3 and was restricted by the propyl paraben concentration.

Results and Discussion

Method optimization

A comparison of the UV absorption spectra (Fig. 2) of methyl paraben, propyl paraben and mebrofenin revealed distinct differences in the shapes of the profiles for the parabens and mebrofenin. The absorption maxima at 256 nm provided the optimum UV wavelength for determining methyl paraben and propyl paraben simultaneously in the presence of mebrofenin. This wavelength maximized the absorbance contributions of both the methyl paraben and propyl paraben while minimizing the interference from the mebrofenin. Examination of the chromatogram for the Choletec placebo, without parabens, (Fig. 3) indicated the absence of any interfering peaks with retention times (t_r) similar to either the methyl or propyl paraben. The mobile phase conditions allowed mebrofenin and other matrix components to be unretained thus resulting in the first eluting peak (t_r = 1.7 min).

A typical chromatogram (Fig. 3) for Choletec revealed baseline resolution for both the methyl paraben (t_r = 2.5 min, k_1' = 0.5) and

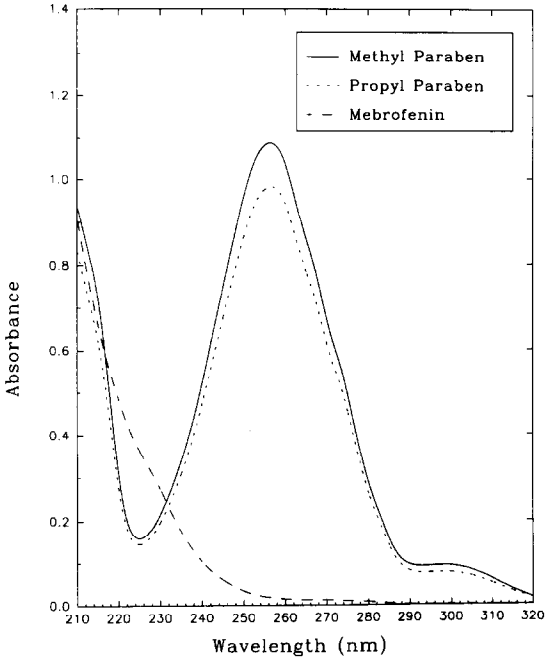

Figure 2
UV spectra of methyl paraben (—), propyl paraben (---) and mebrofenin (– –), from 210 to 320 nm. Conditions: sample in mobile phase, 10 μg ml^{-1} vs mobile phase reference; 1-cm cell.

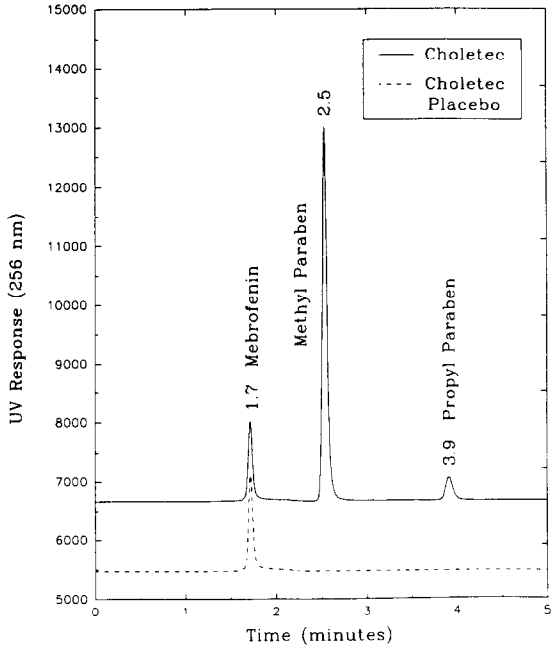

Figure 3
Representative chromatogram of Choletec placebo without methyl and propyl parabens (---) and Choletec (—). Conditions: ODS-Hypersil (250 × 4.6 mm) column; phosphate buffer (pH 6.8, 0.05 M)–acetonitrile–methanol (35:30:35, v/v/v) mobile phase; 1.5 ml min^{-1} flow rate; wavelength of detection 256 nm. Sample: diluted 1:600 with mobile phase.

propyl paraben (t_r = 3.9 min, k_2' = 1.3). Determination of the resolution [$R = 2(t_2 - t_1)/W_1 + W_2$] of the methyl paraben peak from its two adjacent peaks, mebrofenin and propyl paraben, resulted in values of 8 (R_1) and 13 (R_2), respectively.

Method validation

Linearity. The linearity of response for the simultaneous determination of methyl and propyl paraben was evaluated over a one-order of magnitude concentration range (Table 1). Detector response (peak area) versus concentration for methyl paraben was linear from 0.882 to 8.82 μg ml^{-1}, while that for propyl paraben was linear from 0.097 to 0.972 μg ml^{-1}. The disparity in the linear ranges of the two parabens was not a function of the LC method but rather reflected the 9:1 ratio of the methyl and propyl paraben concentrations in the Choletec formulation. The linear range corresponded to a 1:600 dilution of between 12 and 117% of the theoretical vial contents for each of the parabens.

Accuracy. The data for the percentage recovery of methyl and propyl paraben in a spiked Choletec placebo are presented in Table 2. For the range of spiked methyl paraben concentrations corresponding to between 2.71 and 5.03 mg ml^{-1} (60–112% of theoretical), the mean recovery for methyl paraben was 99.2%. Likewise, for the range of spiked propyl paraben concentrations corresponding to between 0.300 and 0.560 mg ml^{-1} (60–112% of theoretical), the mean recovery for propyl paraben was 98.8%. These data reflect that there are good recoveries for both of the parabens well beyond the normal concentrations expected in the product.

Precision. The assay measurements of 10 replicates from a single vial of Choletec showed good reproducibility for both methyl and propyl paraben with average values of 4.58 ± 0.02 and 0.500 ± 0.009 mg ml^{-1}, respectively. With RSD values of 0.46 and 1.7% for methyl and propyl paraben, respectively, the method exhibited a very acceptable level of intra-assay precision. In addition, the ratio of methyl–propyl paraben which should have a theoretical value of 9.0, was found to be 9.2 ± 0.1.

Limit of detection. The ratio of methyl–propyl paraben in the formulation had a direct effect on the limit of detection of the method. The limits of detection at a signal-to-noise of 3 were found to be 0.9:0.1 μg ml^{-1} for the methyl–propyl paraben combination and were restricted by the lower concentration of propyl paraben.

Ruggedness and system suitability. Two ODS–Hypersil columns of differing lots from the same manufacturer were examined to evaluate the ruggedness of the LC method and define criteria for system suitability. The data

Table 1
Linear regression analysis for the simultaneous HPLC determination of methyl paraben and propyl paraben

Analyte	Linear range (μg ml^{-1})	Slope	*y*-Intercept (area units)	Correlation coefficient
Methyl paraben	0.882–8.82	7006	440	0.9999
Propyl paraben	0.097–0.972	6143	−43	0.9999

Table 2
Simultaneously determined percentage recoveries of methyl paraben and propyl paraben in spiked Choletec preparations

Methyl paraben			Propyl paraben		
Spiked conc. (mg ml^{-1})	Measured* conc. (mg ml^{-1})	Recovery (%)	Spiked conc. (mg ml^{-1})	Measured* conc. (mg ml^{-1})	Recovery (%)
5.03	5.13	102	0.560	0.572	102
4.51	4.39	97.3	0.500	0.491	98.2
4.01	3.96	98.8	0.445	0.434	97.5
2.71	2.68	98.9	0.300	0.292	97.3
Mean		99.2			98.8
RSD (%)		2.0			2.2

*Reported concentration values are an average of n = 2 determinations.

Table 3
Performance testing comparing two ODS-Hypersil columns from the same manufacturer

	Methyl paraben			Propyl paraben		
Column No.	k'_1	R_1	n_1	k'_2	R_2	n_2
SN07578	0.5	8	9,000	1.3	13	10,000
SN93040798	0.5	8	14,000	1.3	13	13,000

k'_1 = Capacity factor for methyl paraben.
R_1 = Resolution of the methyl paraben and mebrofenin peaks.
n_1 = Number of theoretical plates for methyl paraben.
k'_2 = Capacity factor for propyl paraben.
R_2 = Resolution of the methyl paraben and propyl paraben peaks.
n_2 = Number of theoretical plates for propyl paraben.

presented in Table 3 reflect excellent column-to-column performance in terms of capacity factor (k') and resolution (R). In addition, no significant changes were noted in either the retention times or R_1 and R_2 values of the parabens after over 100 injections on a single column.

Based upon this experience, the criteria for a satisfactory system suitability were established at an R_2 value >2 and a RSD of ≤2% for five replicate injections of the second combination working standard (6.6 μg methyl paraben, 0.7 μg propyl paraben ml^{-1}).

Conclusions

The simultaneous determination of methyl and propyl paraben in the presence of a high concentration of mebrofenin (45 mg ml^{-1}) can be performed by a rapid (<5 min), direct, reversed-phase LC method. Detection at 256 nm minimized the UV contribution attributed to mebrofenin while maximizing the response for the parabens. The method was linear over a one-order of magnitude concentration range, corresponding to a 1:600 dilution of between 12 and 117% of the theoretical vial contents for each of the parabens. In addition, the method was shown to be reproducible, accurate, rugged and devoid of matrix interferences.

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