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First-derivative UV spectrophotometric and high-performance liquid chromatographic analysis of some thiazide diuretics in the presence of their photodecomposition products

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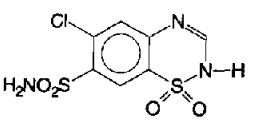
Abstract: Ethanolic solutions of three thiazide diuretics, chlorothiazide, hydrochlorothiazide and trichlormethiazide, were irradiated with a high-pressure mercury lamp. The products were isolated and their first-derivative UV spectra in ethanol were recorded and compared to those of the parent compounds. The determination of the parent compounds in the presence of the decomposition products was carried out at wavelengths near 220 nm using the zero-crossing technique. Three reversed-phase HPLC methods were also developed for the analysis of the parent compounds. In parallel analyses of the reaction mixtures a good correlation was achieved between these two methods in the determination of hydrochlorothiazide and trichlormethiazide while there was greater variation in the results of chlorothiazide.

Keywords: *Thiazide diuretics; photodecomposition; analysis; first-derivative UV spectrophotometry; HPLC.*

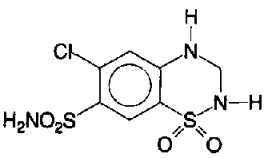
Introduction

Derivative UV spectrophotometry has been successfully applied to the determination of decomposition rates of drugs in the presence of their acid- and alkaline-induced decomposition products by measuring the derivative values of the solutions at the wavelength of zero contribution of the products [1, 2]. In photodecomposition reactions of the thiazide diuretics several products are normally formed [3]. They often have similar UV spectra, with their maxima shifted to shorter wavelengths, compared to the parent compound. In the 210–230 nm region, the difference between the maxima of the product mixture and of the parent compound in ethanolic solution may be about 5–15 nm. Thus the zero-crossing points of the corresponding first-derivative (D_1) spectra have the same wavelength difference and it is possible to find a point where the first derivative of the product mixture is practically zero and that of the parent compound close to the maximum. The aim of this work was to study the suitability of first-derivative spectrophotometry for monitoring photodecomposition reactions of the thiazides in ethanolic solution in the presence of small amounts of decomposition products using HPLC as a reference method. Three chlorine-containing members of the group chlorothiazide (CT), hydrochlorothiazide (HCT) and trichlormethiazide (TCMT) were chosen as model compounds.

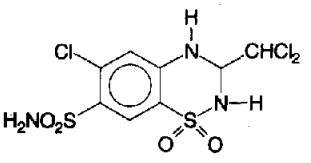
CT



HCT



TCMT



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Experimental

Materials

CT was kindly supplied by Huhtamäki Oy (Turku, Finland) and HCT by Orion Oy (Espoo, Finland). TCMT and the internal standard hydroflumethiazide (HFMT) for HPLC were purchased from Sigma (St Louis, MO). The identity and purity of the compounds were verified by IR spectrometry, TLC and melting points. HPLC-grade organic solvents (Rathburn, Walkerburn, Scotland) and water and Suprapur acetic acid (Merck AG, Darmstadt, Germany) were used in the preparation of the mobile phases and 96% ethanol (Oy Alko Ab, Helsinki, Finland) as the solvent for the parent compounds. All other chemicals were of analytical-reagent grade.

Apparatus

The source of irradiation was a high-pressure mercury lamp (Original Hanau TQ 718 at 500 W) equipped with a quartz glass cooling mantle. The UV and first-derivative spectra were recorded with a Philips PU 8740 UV–vis spectrophotometer using 10-mm quartz cells. The wavelength range was 200–300 nm, the spectral bandwidth 1 nm and the scan speed 125 nm min^{-1}. The apparatus utilized in the HPLC analyses consisted of a Waters 501 pump, Rheodyne 7125 injector with a 20-μl loop, Waters 991 photodiode array detector and a NEC Powermate 386/25 computer with Waters PDA software. The IR spectra were recorded with a Pye–Unicam SP3-200 infrared spectrophotometer, using the KBr pellet technique, and the melting points determined with an Electrothermal digital melting point apparatus.

Isolation of the decomposition products

Ethanolic solutions (0.5 mM) of the parent compounds were irradiated in a 20-mm quartz cuvette (volume 7 ml) for 20 min at ambient temperature. The distance from the lamp was 25 cm for HCT and TCMT and 10 cm for CT because of greater photostability of the last-named compound. The irradiated solutions were evaporated to a small volume under reduced pressure and applied as a line to an aluminium sheet precoated with silica gel 60 F_{254} (Merck). After the development of the sheet using ethyl acetate as the eluent, the areas containing products were scratched off and shaken with acetone. Acetone was used in order to minimize the dissolution of the silica gel.

After evaporation of the acetone the products were dissolved in ethanol. The solutions were diluted, if necessary, and their zero- and first-order spectra recorded and compared to those of the parent compounds.

Irradiation of the solutions for quantitative analysis

The 0.5 mM ethanolic solutions of the parent compounds were irradiated at ambient temperature in a 20-mm quartz cuvette (volume 7 ml) containing a small magnetic bar on a stand built on a magnetic stirrer (Thermolyne Nuova II) (Fig. 1). The 313 nm line from the radiation of the lamp was isolated with a potassium chromate solution (5×10^{-4} M in a 10-mm quartz cuvette) and a Corning colour filter CS-7-54 [4]. The distance of the stand from the lamp was varied to control the extent of the reaction between about 0 and 50%. At intervals of 30 min the lamp was switched off and a 0.5-ml sample for HPLC was taken from the reaction vessel, the internal standard solution added (0.5 ml) and the solution diluted to 5.0 ml with the appropriate mobile phase. Another sample of 0.5 ml for the spectrophotometric analysis was diluted 10-fold with ethanol. The potassium chromate solution was replaced with a fresh one and the lamp was restarted after 10 min.

Spectrophotometric analysis

The compounds investigated have UV absorption maxima and zero-crossing points in their D_1 spectra in the regions 220–230 nm and 265–280 nm. In the latter region the difference between the zero-crossing points of first order spectra of HCT and TCMT and of their decomposition products is only about 2 nm. The corresponding difference in the spectra of CT and the decomposition products is about 10 nm. However, their UV spectra are broad and therefore the corresponding D_1 values small. The results obtained by derivative spectrophotometry were systematically higher than those obtained by the HPLC method when the concentration of the parent compound was measured at 271 nm, the zero-crossing point of the main product. Therefore, the measurements were made using maxima near 220 nm where the D_1 values are larger. The zero-crossing point of the D_1 spectrum of

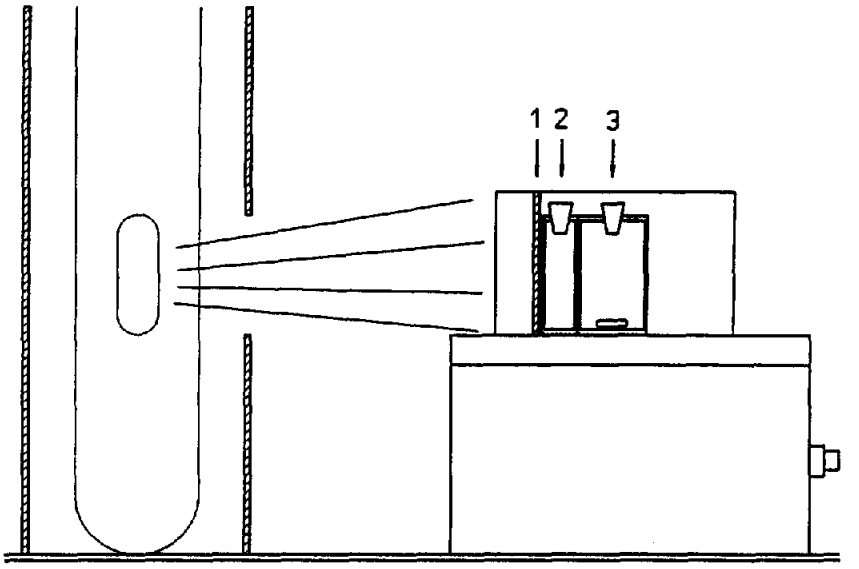

Figure 1
Schematic representation of the irradiation apparatus used. (1) Corning filter CS-7-54; (2) potassium chromate solution; (3) reaction cuvette.

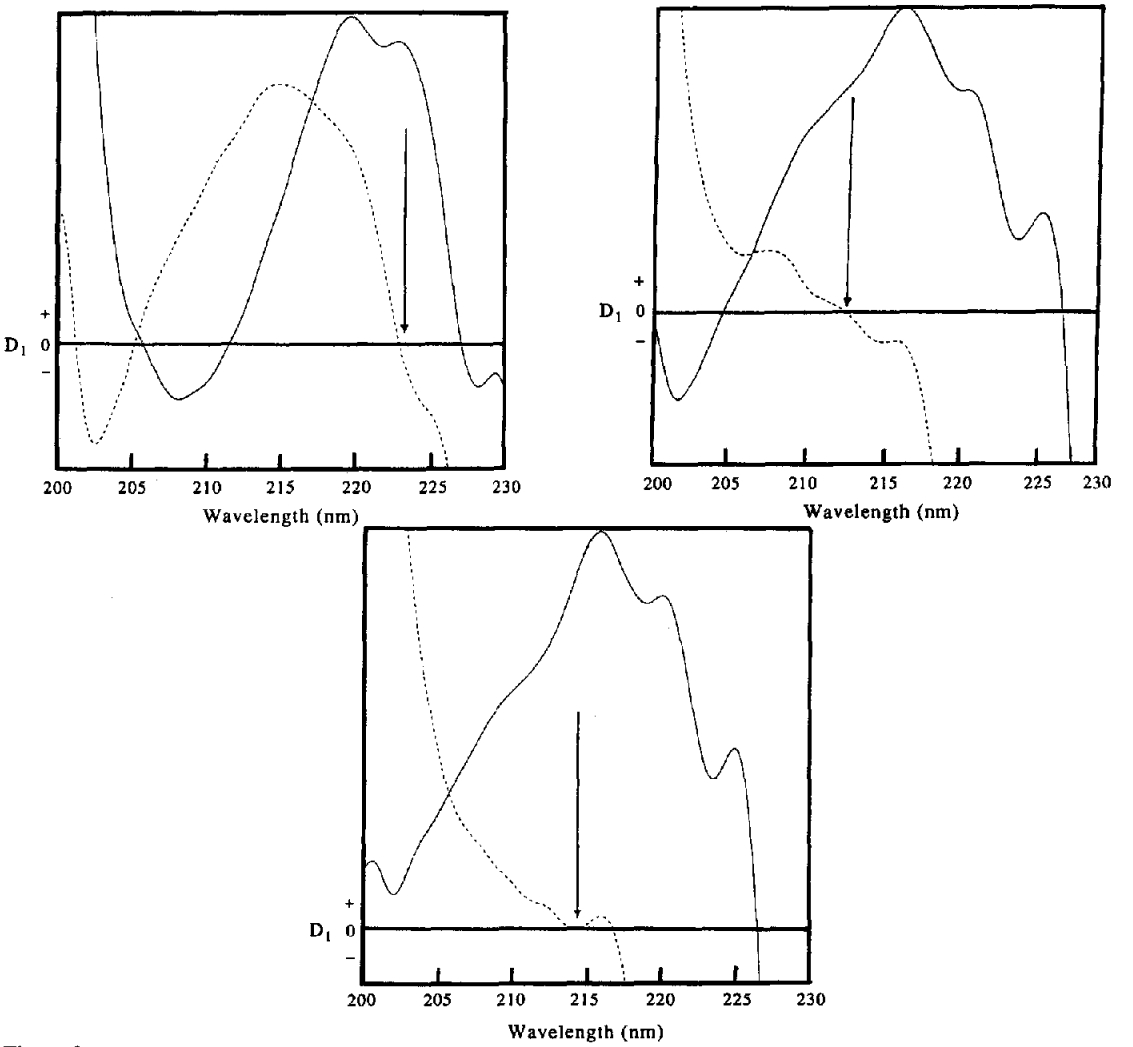

Figure 2
First-derivative UV spectra of the parent compounds (solid lines) and of their photodecomposition products (dotted lines). (a) CT, (b) HCT, (c) TCMT.

CT is 223 nm and the corresponding points in the D_1 spectra of HCT and TCMT are 213 and 214 nm, respectively (Fig. 2). The concentrations of the parent compounds were calculated based on the D_1 values of the irradiated solutions at these wavelengths compared to the initial solutions.

The calibration graphs covering the concentration range 1.0–5.0 × 10^{-5} M were obtained by transferring 0.1–0.5 ml of the 0.5 mM solutions of the parent compounds into a 5.0-ml volumetric flask and diluting to volume with ethanol. The D_1 amplitudes were plotted against the corresponding concentrations. The precision of the method was studied by diluting 0.5 ml and 0.3 ml of the 0.5 mM solutions of the parent compounds to 5.0 ml six times.

HPLC analysis

The method development was carried out according to Snyder *et al.* [5]. In the analysis of photolysed solutions of CT and TCMT a Hibar LiChrospher RP-18 column (125 mm × 4 mm i.d., Merck particle size 5 μm) was used. The mobile phase was acetonitrile–water–acetic acid (10:88:2, v/v/v). The flow rate was 1.0 ml min^{-1} for the analysis of CT while a higher rate (1.7 ml min^{-1}) was used for TCMT to obtain a reasonable analysis time. For the determination of HCT no satisfactory separation between the main products was achieved with this column though the mobile phase was modified by trying methanol and tetrahydrofuran and mixtures of acetonitrile, methanol and tetrahydrofuran as the organic modifier. The products were separated by using a longer RP-18 column (Hibar LiChrosorb 250 mm × 4 mm i.d., Merck, particle size 5 μm) and 2.5% tetrahydrofuran as the organic modifier. A flow rate of 2.0 ml min^{-1} was used. HFMT proved to be the best internal standard of several compounds tested for the three thiazides and was used in a concentration of 0.5 mM. The sample solutions were injected twice (20 μl using the full loop) and the concentrations of the parent compounds were calculated based on the peak area ratios (analyte/internal standard) in the samples and in the initial solutions. The absorption maxima of the parent compounds in the mobile phases were chosen as detector wavelengths, *viz* 280 nm, 271 nm and 269 nm for CT, HCT and TCMT, respectively.

The calibration graphs covering concentration range 1.0–10.0 × 10^{-5} M were obtained by transferring 0.1–1.0 ml of the 0.5 mM solutions of the parent compounds and 0.5 ml of the 0.5 mM internal standard solution into a 5.0-ml volumetric flask and diluting to volume with the mobile phase. The peak area ratio (analyte/internal standard) was plotted against the concentration of the analyte. The precision of the method was studied by diluting 0.5 ml and 0.3 ml of the 0.5 mM solutions of the parent compounds and the internal standard solution to 5.0 ml six times.

Results and Discussion

Typical chromatograms from HPLC analyses of the irradiated solutions are presented in Fig. 3. The parent compounds and the internal standard are well separated from the main products. The plots of the results obtained by the HPLC method compared to those obtained by the first-derivative method on the same irradiated solutions calculated in percentages of the initial concentrations are shown in Fig. 4. The results for these two methods correlate relatively well with each other in the analyses of HCT and TCMT. The correlation coefficient of the regression line for HCT is 0.987 and the slope 0.946. For TCMT the corresponding values were 0.983 and 1.029. In the results for the analysis of CT greater variation was observed especially at concentrations below 85% of the initial concentration. The correlation coefficient calculated from all the results presented is 0.918 the slope being 0.759. Both methods showed linear behaviour throughout the concentration ranges studied (Table 1). The precision of the HPLC methods was better with relative standard deviations (RSD) between 0.72 and 0.96% compared to the values between 0.80 and 2.50% obtained by derivative spectrophotometry. The largest RSD values were typically found in the spectrophotometric analyses of CT solutions. The results of this study show that first-derivative spectrophotometry can be used as a simple and rapid alternative analytical method for monitoring the disappearance of the parent compounds at early stages of the photolysis of HCT and TCMT in ethanolic solution at the zero-cross-ing points of the product mixtures at wavelengths as low as 213–214 nm. However, determination of the products in the photolysed solutions is more difficult than by HPLC. The source of the variation in the results for

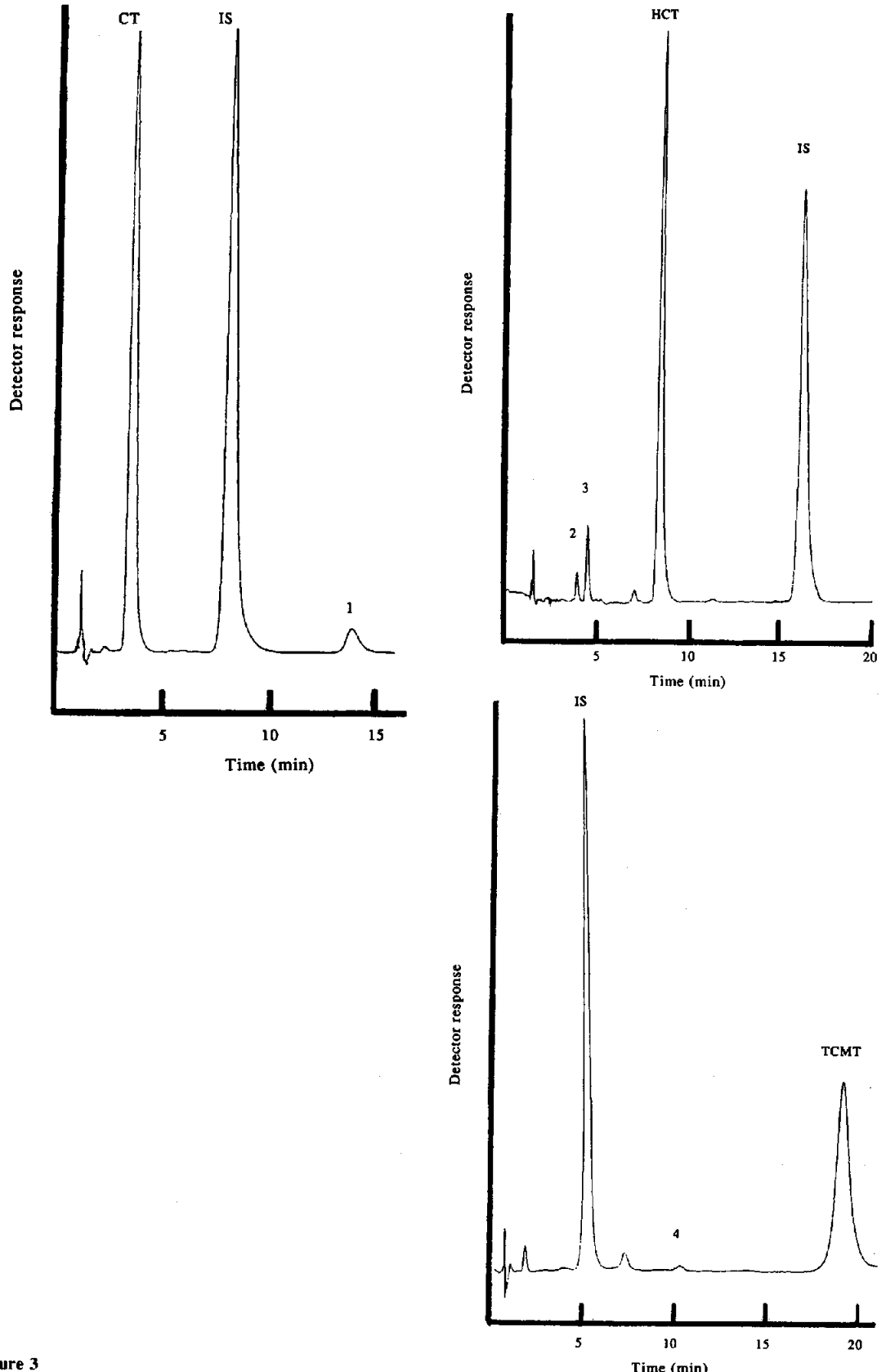

Figure 3
Typical chromatograms of the irradiated solutions. (A) Mobile phase acetonitrile–water–acetic acid (10:88:2, v/v/v) 1.0 ml min^{-1}; (B) mobile phase tetrahydrofuran–water–acetic acid (2.5:95.5:2, v/v/v) 2.0 ml min^{-1}; (C) mobile phase acetonitrile–water–acetic acid (10:88:2, v/v/v) 1.7 ml min^{-1}. IS = internal standard (HFMT), 1,2,3 and 4 are the main decomposition products.

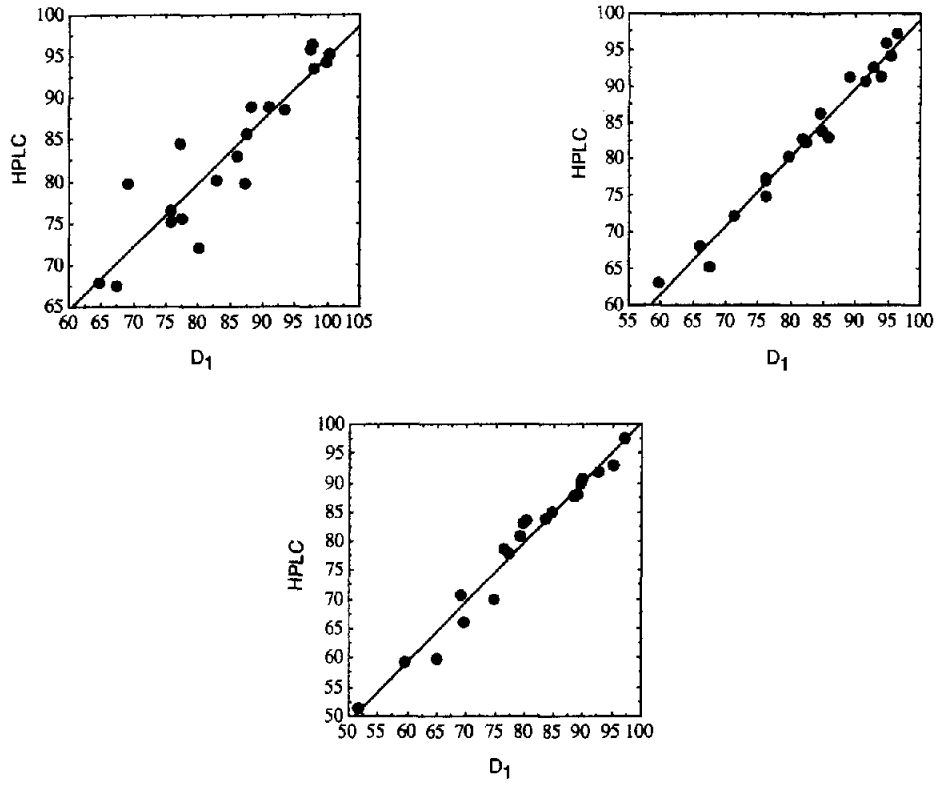

Figure 4
Correlation between the residual percentages as determined by HPLC and first-derivative method. (a) CT, (b) HCT, (c) TCMT.

Table 1
Calibration graphs of the parent compounds

Method	Compound	Slope	Intercept	Correlation coefficient	Conc. range ($\times 10^{-5}$ M)
D_1	CT	11.21	−2.46	0.9997	1.0–5.0
D_1	HCT	13.49	−2.08	0.9998	1.0–5.0
D_1	TCMT	19.18	−3.11	0.9997	1.0–5.0
HPLC	CT	0.1563	0.0118	0.9998	1.0–10.0
HPLC	HCT	0.2095	−0.0229	0.9997	1.0–10.0
HPLC	TCMT	0.2069	−0.0276	0.9999	1.0–10.0

the analysis of CT requires further study. However, in later experiments it was found that second-derivative spectrophotometry is not a suitable method for the analysis of CT because of poor precision at wavelengths where the products have zero contribution.

References

[1] H. Abdellatef, M. Elbalkiny and A. Aboulkheir, *J. Pharm. Biomed. Anal.* **7**, 571–576 (1989).

[2] M. Bedair, M. Korany, M. Ebdel-Hay and A. Gazy, *Analyst* **115**, 449–453 (1990).

[3] V. Ulvi and S. Tammilehto, *Acta Pharm. Nord.* **1**, 195–200 (1989).

[4] D. Nicodem and O. Aquilera, *J. Photochem.* **21**, 189–193 (1983).

[5] L. Snyder, J. Glajch and J. Kirkland, *Practical HPLC Method Development*, p. 228. Wiley, New York (1988).

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