A NEW CONCEPT FOR TESTING CHROMATOGRAPHIC PEAK PURITY: APPLICATION TO DETERMINATION OF THE MAIN INGREDIENTS IN TEA EXTRACTS

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Abstract

A new concept was applied to test the spectral purity of chromatographic peaks. The proposed concept is based on the application of the orthogonal functions (P1 and P2) to the absorption spectra recorded at five different time intervals throughout the eluted chromatographic peak. Ratios of strong peaks of the P1 and P2 convoluted absorption spectra were computed for the investigated samples and for standard solutions of the corresponding reference materials. Such ratios are considered spectrophotometric characteristics for each compound, independent of concentration. The percentage deviation in these ratios between the samples and the reference materials exceeded 2% in case of impure peaks. For further assessing the peak purity, the obtained convoluted curves were examined. The pure peaks share the same characteristic spectral features and intersect at the same wavelengths. This new concept was applied to determine the peak purity of the main alkaloids in black, green and white tea using the Agilent chromatography software. The purity factor of TB peak exceeds the calculated threshold limit indicating that the eluted CF peak is pure. The purity factor of TB peak exceeds the calculated threshold limit indicating that the eluted peak of TB in HPLC peaks of CF, TP and TB reference standard solutions and standard mixture were tested using Agilent chromatography software. The purity factor is within the calculated threshold limit indicating that the eluted peaks are pure. The chromatograms of black, green and white tea extracts showed peaks corresponding to CF and TB obtained using the specified chromatographic conditions (see experimental).

Results & Discussion

Introduction

The spectrachromatograms extracted at different time intervals across an HPLC peak are UV absorption spectra of the eluted compounds. The chromatographic peaks are dependent on the structure of the eluted compound while the intensity of the absorption is dependent on the concentration of such compound at any time throughout the elution of the HPLC peak. The convolution of an absorbing compound and/or impurity along with the main compound would contribute to the absorption of the main compound. Ratios of strong peaks of P1 and P2 convoluted absorption spectra are considered spectrophotometric characteristics of each compound, independent of concentration and these ratios could be applied to assess chromatographic peak purity.

Experimental

The proposed method for testing peak purity was applied. The results indicated that the eluted peak of TB in HPLC extracts of teas samples is impure due the the following observations:

1. The spectrachromatograms extracted at different time intervals throughout the eluted peak are similar to each other.
2. The UV-Vis absorption spectra extracted at different time intervals of the elution of each peak intersect at the same wavelengths (Fig. 1).
3. The UV-Vis absorption spectra extracted at different time intervals of the elution of each peak intersect at the same wavelengths (Fig. 1).
4. The ratios of the absorbance and orthogonal (p1 and p2) optima of the UV-Vis absorption spectra extracted at different time intervals of the eluted peak are similar to those of standard CF with RSD % values less than 2% (Table 1).

Fig. 1. Convoluted orthogonal absorption curves obtained at five time intervals across the eluted chromatographic peaks of theophylline and caffeine in green tea extract

Table 1. Ratios of the orthogonal optima (p1 and p2) of the spectrachromatograms extracted at different time intervals for the eluted CF peak obtained from HPLC of the extract of green tea sample

Table 2. Ratios of the orthogonal optima (p1 and p2) of the spectrachromatograms extracted at different time intervals for the eluted CF peak obtained from HPLC of the extract of green tea sample

Fig. 2. Purity plots of TB and CF peaks obtained from HPLC of green tea sample showing the autothreshold line (= ) and the purity line ( )

Chemical & Reagents

Pharmaceutical grades of CF, TP and TB were kindly supplied by Amriya for pharmaceutical industries, Alexandria, Egypt. Acetonitrile (HPLC grade) was purchased from Merck. Sodium acetate, glacial acetic acid, anhydrous sodium sulphate, lead acetate and sodium carbonate were all of analytical grade. Tea samples were purchased from a local market.

Preparation of tea extract

Samples of 1 g of black, green and white tea were accurately weighed and boiled for 30 min in 100 mL of distilled water. Water evaporation was compensated during boiling. The obtained infusions were then filtered and the filtrate was quantitatively transferred into a 100-mL volumetric flask and diluted to volume with water. A 0.5 mL aliquot of lead acetate (10 % w/v) was added to a 25 mL aliquot of the sample to precipitate tannins and the mixture was stirred for 3 min at room temperature. The solutions were filtered and 0.1 mL of sodium carbonate (10 % w/v) was added to the filtrate to remove excess lead acetate. The mixture was stirred for 2 minutes at room temperature then filtered and volumes were adjusted to 100 mL with distilled water.

Chromatographic conditions

Aliquots of standard CF, TP and TB solutions containing 15 µg mL−1 were injected into the HPLC column concurrently with the tea extracts using the chromatographic conditions described in the BP under the monograph of TB on "P tatto for related substances". The chromatograms were extracted at 272 nm and integrated using Agilent ChemStation software.

Instrument

Agilent high performance liquid chromatographic system (1200 series) comprised of a quaternary pump with rheodyne 7725i 7-port sample injector and photodiode array detector system controlled by Agilent chemstation software for LC.

References