Development and validation of spectrophotometric and high-performance thin-layer chromatographic methods for the determination of folic acid in the presence of its impurities (degradation products)

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Three accurate, sensitive, simple, and precise spectrophotometric methods along with thin-layer chromatography (TLC)–densitometric method were developed, optimized, and validated for the determination of folic acid in the presence of its two impurities (photodegradation products), namely, pteroic acid and *para*-aminobenzoic acid. Method A is the ratio difference spectrophotometric method (RDSM) which depends on measuring the difference value in the ratio spectrum, where the difference between 291 and 313 nm was used for the determination of folic acid, while the difference between 305 and 319 nm was selected for the estimation of *para*-aminobenzoic acid; on the other hand, pteroic acid can be determined using the first derivative of ratio spectra spectrophotometric method at 262 nm. Method B is the double-divisor spectrophotometric method (DDSM); this method is based on using the ratio spectrum obtained by the division of the spectrum of ternary mixture by the spectrum of binary mixture containing two of the three mentioned components, and in this method, folic acid, *para*-aminobenzoic acid, and pteroic acid were measured at 242, 313, and 258 nm, respectively. Method C is the mean-centering of ratio spectra spectrophotometric method (MCR); in this method, folic acid, *para*-aminobenzoic acid, and pteroic acid can be measured using the mean-centered second ratio spectra amplitudes at 317–318 (peak to peak), 264–265 (peak to peak), and 232 nm, respectively. Lastly, method D is a TLC–densitometric one that depends on the separation and quantification of the mentioned components on TLC silica gel 60 F_{254} plates, using methanol– iso-propanol–water–acetic acid (9:0.5:0.5:0.2, by volume) as the developing system, followed by densitometric measurement of the separated bands at 280 nm. Method validation was carried out according to the International Conference on Harmonisation (ICH) guidelines, and the proposed methods were successfully applied to the analysis of folic acid in pharmaceutical formulations, where no interference from additives has been found. The results obtained by the proposed methods were statistically compared with those obtained by the official reversed-phase high-performance liquid chromatography (RP-HPLC) method, in which no significant difference was observed.

Keywords:
Folic acid; Pteroic acid; para-Aminobenzoic acid; Ratio difference; Double divisor; Mean-centering; Thin-layer chromatography–densitometric method