

HPLC/DAD DETERMINATION OF ALOIN BESIDES FLAVONOIDS IN COMPLEX PLANT PHARMACEUTICALS

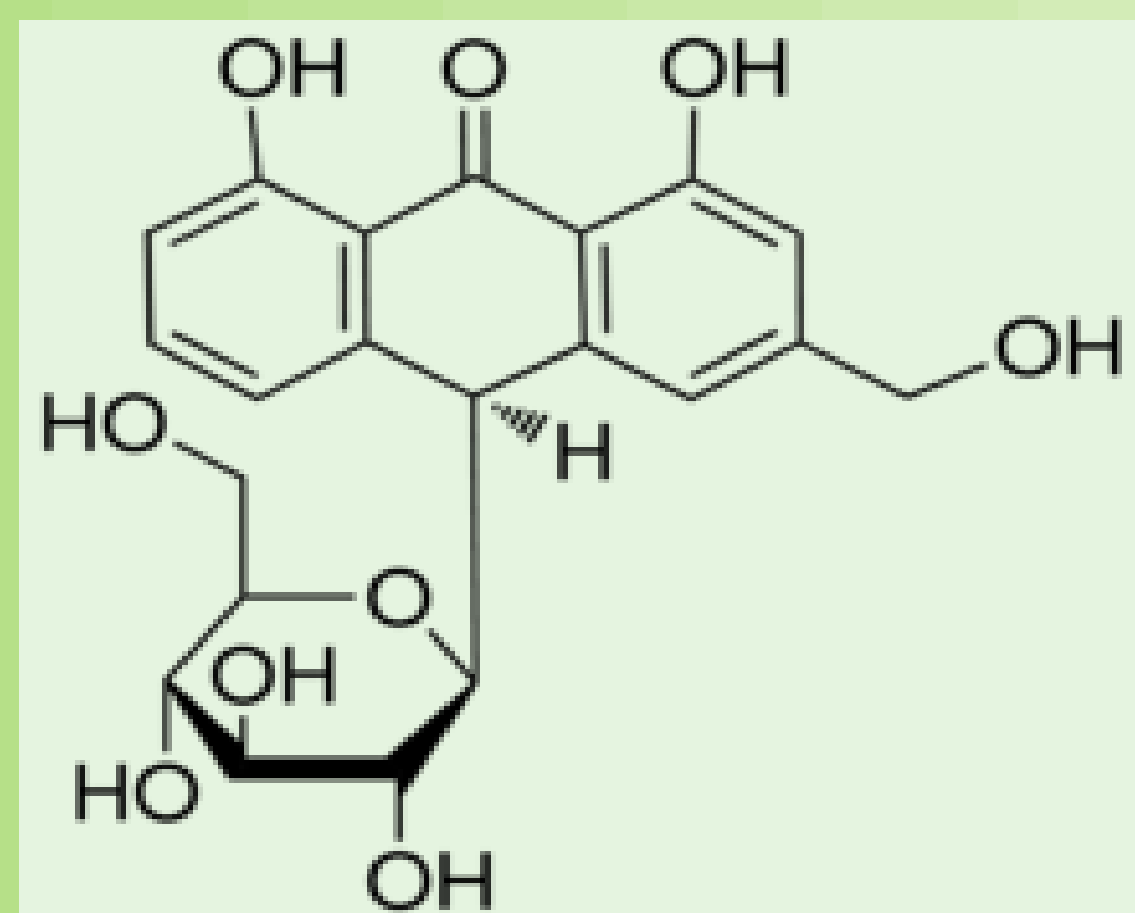


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INTRODUCTION

A simple HPLC/DAD method for determination of aloin in complex matrices was proposed, using aloin A as a marker compound. For this method the obtained validation results are in satisfactory ranges. The method is successfully applied for analysis of aloin in *Aloe barbadensis* Mill. plant dried exudate and pharmaceuticals which besides this exudate contain mixture of different extracts from: *Achillea Millefolium* L., herba; *Calendula officinalis* L., flos; *Cornus mas* L., plant cortex; *Cynodon dactylon* L., rhizome; *Hypericum perforatum* L., herba; *Inula helenium* L., radix and *Viscum album* L., herba. It was shown that other present compounds such as flavonoids (quercetin, hyperozid and rutin) do not interfere in determination and they may be analyzed simultaneously (Table 1). This method is proposed to be used in control of pharmaceuticals.



Aloin A



Aloe barbadensis Mill.

RESULTS

It was shown that other present compounds such as flavonoids (quercetin, hyperozid and rutin) do not interfere in determination and they may be analyzed simultaneously (Table 1). This method is proposed to be used in control of pharmaceuticals.

Table 1. Chromatographic and spectroscopic data obtained by using optimized gradient HPLC/DAD method

| Compound | t_r (min) | k' | R_s | S | A_{max} (nm) |
|-----------|-------------|-------|-------|------|--------------------|
| Rutin | 8.6 | 6.41 | | 1.04 | 200; 255; 355 |
| Hyperozid | 9.3 | 7.02 | 2.76 | 1.06 | 200; 255; 355 |
| Aloin A | 15.0 | 11.93 | 22.20 | 0.94 | 200; 268; 295; 355 |
| Quercetin | 19.4 | 15.64 | 15.24 | 0.91 | 200; 255; 375 |

t_r – retention time; k' – retention factor; R_s – resolution; S – peak's symmetry;
 A_{max} – wavelength at the maximum absorption

VALIDATION

Table 2. Validation results obtained by linearity test and LOD/LOQ test

| Test | Parametar | Aloin A | Hyperozid | Rutin | Quercetin |
|---|----------------|--|---|--|--|
| I. Linearity test | Range (µg /ml) | 0.67 - 84.00 | 1.13 - 84.60 | 1.13 - 84.00 | 1.60 - 200.00 |
| | equation | $A = 1.1391 m + 3.9503$ | $A = 1.7646 m + 3.8679$ | $A = 1.3688 m + 3.0676$ | $A = 2.6233 m + 4.392$ |
| | SD | 7.4343 | 11.7228 | 7.9145 | 37.7369 |
| | R^2 | 0.9996 | 0.9997 | 0.9997 | 0.9997 |
| II. a LOD/LOQ test: Results obtained by testing low concentration range | Range (µg/mL) | 0.0336 – 0.336 | 0.0564 – 0.564 | 0.056 - 0.560 | 0.080 – 0.800 |
| | equation | $A = 1.1914 m + 0.1761$ | $A = 1.6138 m + 0.4041$ | $A = 1.229 m + 0.3815$ | $A = 3.4383 m + 1.2978$ |
| | SD | 0.1142 | 0.2060 | 0.3183 | 1.1334 |
| | R^2 | 0.9967 | 0.9979 | 0.9914 | 0.9931 |
| II. b LOD/LOQ test: Results obtained by S/N | LOD/LOQ | 0.32 ng/0.96 ng 32/96 ng/mL | 0.42 ng/1.28 ng 42/128 ng/mL | 0.85 ng/2.59 ng 85/259 ng/mL | 1.09 ng/3.30 ng 109/330 ng/mL |
| | LOD/LOQ | 0.33 ng/0.99 ng 33.00 ng/mL /99.00 ng/mL | 0.10 ng/0.30 ng 9.79 ng/mL /29.68 ng/mL | 0.12 ng/0.37 ng 12.00 ng/mL /37.00 ng/mL | 0.30 ng/0.91 ng 29.88 ng/mL /90.57 ng/mL |

Table 3. Data obtained by accuracy test (n = 3)

| Compound | Concentration (γ_1 , µg/ml) | | Recovery | | Concentration (γ_2 , µg/ml) | | Recovery | | Concentration (γ_3 , µg/ml) | | Recovery | |
|-----------|-------------------------------------|-------|----------|--|-------------------------------------|-------|----------|--|-------------------------------------|-------|----------|--|
| | expected | found | %, m/m | | expected | found | %, m/m | | expected | found | %, m/m | |
| aloin A | 68.46 | 70.36 | 102.76 | | 66.08 | 70.14 | 106.14 | | 62.13 | 63.52 | 102.24 | |
| | 5.68 | 5.33 | 93.84 | | 5.22 | 4.922 | 94.25 | | 4.91 | 4.00 | 81.47 | |
| hyperozid | 9.38 | 9.62 | 102.56 | | 8.63 | 8.80 | 101.97 | | 8.13 | 7.82 | 96.19 | |
| rutin | 9.75 | 9.31 | 99.25 | | 9.40 | 9.11 | 96.91 | | 9.17 | 9.29 | 101.31 | |
| quercetin | 15.84 | 16.03 | 101.20 | | 13.79 | 12.82 | 92.97 | | 12.43 | 12.08 | 97.18 | |

EXPERIMENTAL

The chromatographic separation was achieved on C18 HPLC column, mobile phase composed of acetonitrile and water (pH 2.6) in gradient mode, flow-rate 1.5 ml/min, with simultaneous UV detection at 295 nm for aloin, and 255 nm and 375 nm for flavonoids.

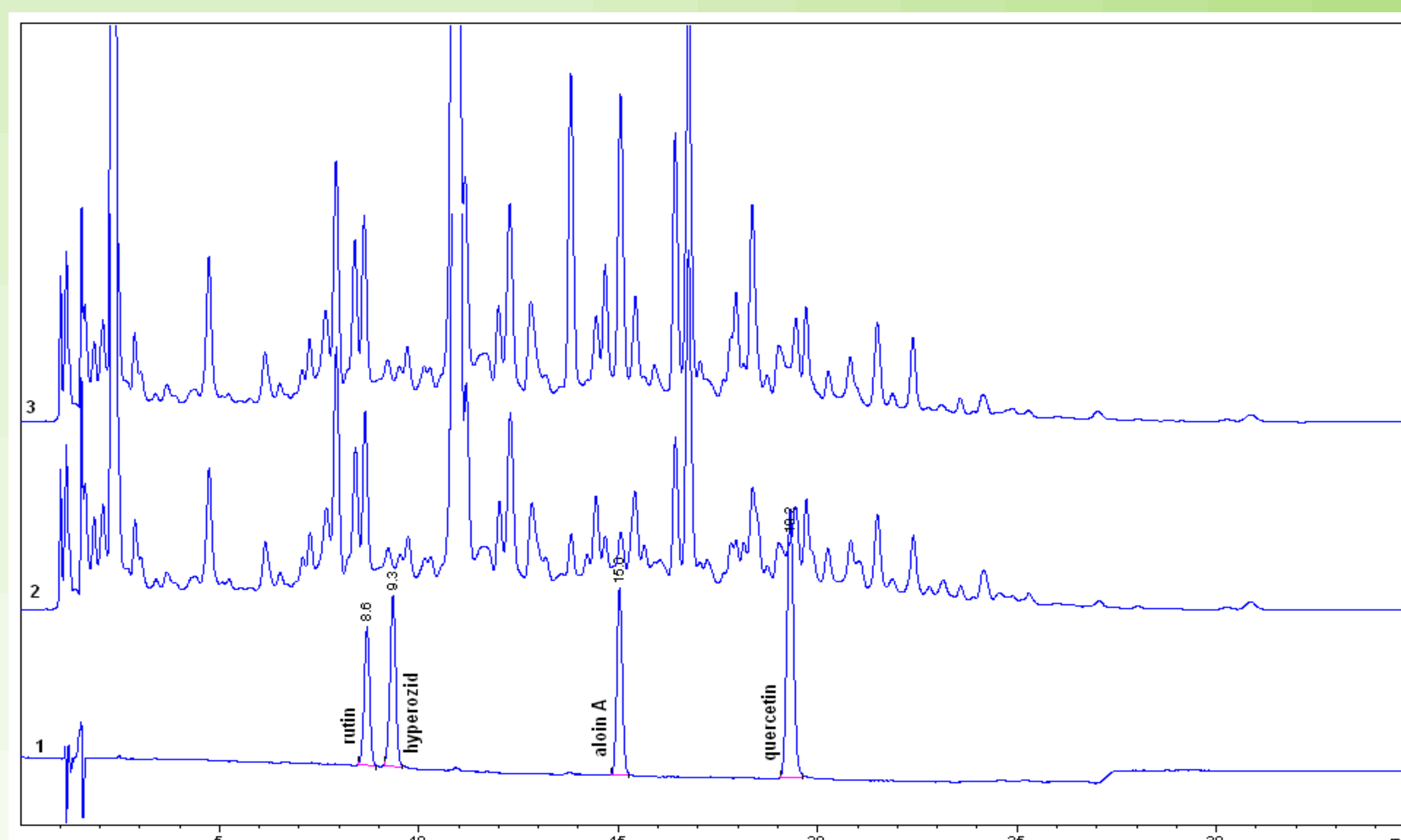


Fig. 1. Chromatograms scanned at 295 nm: (1) mixture solution of standard substances (rutin, hyperozid, aloin A and quercetin), (2) pharmaceutical sample 1, (3) pharmaceutical sample 2

IDENTIFICATION

The identification of aloin A and flavonoids was done by comparison of retention times of components (Fig. 1), UV spectra of their peaks with the same retention (Fig. 2) and by standard addition method for aloin A (Fig. 3).

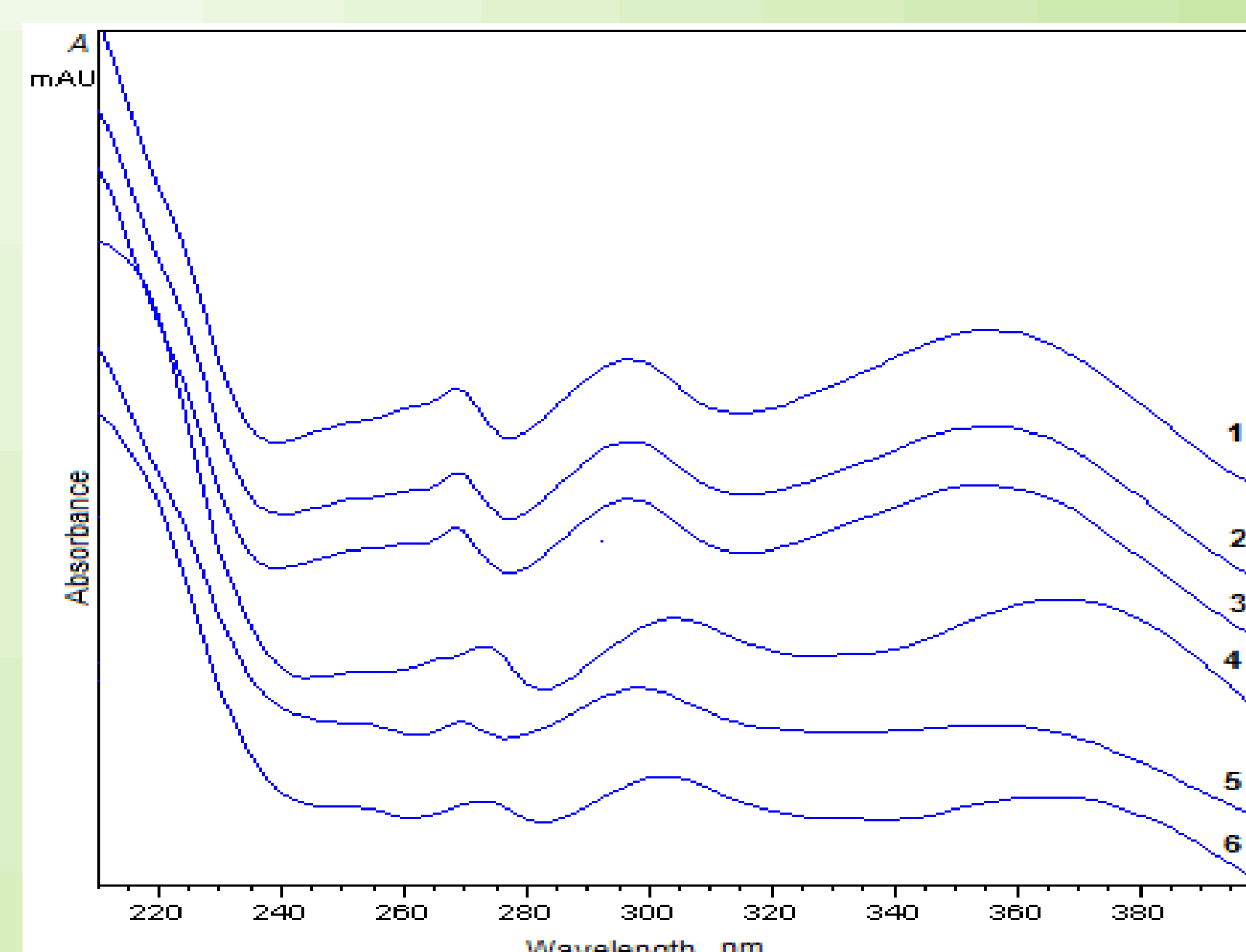


Fig. 2. Identification of aloins at real complex samples mixtures by comparison of UV spectra: (1) aloin A peak ($t_r=15.0$ min) from the reference standard solution and peaks of compounds identified at sample 2 which contains *Aloe Vera* dried exudate: (2) aloin A ($t_r=15.0$ min); (3) aloin B ($t_r=13.8$ min); (4) aloin-like compound ($t_r=16.4$ min) and peaks of compounds identified at sample 1 which also contains *Aloe Vera* powder extract: (5) aloin A ($t_r=15.0$ min); (6) aloin B ($t_r=14.6$ min)

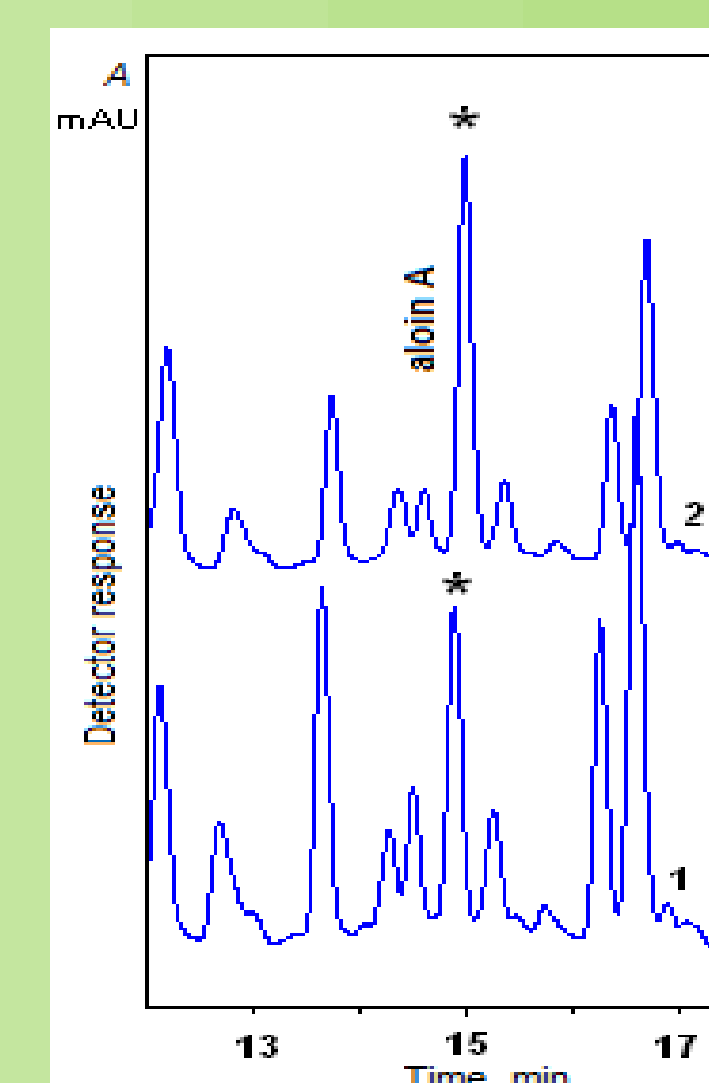


Fig. 3 Identification of aloin A at a real complex sample mixture by addition of standard aloin A compound to sample 2: (1) chromatogram of sample 2, (2) chromatogram of sample 2 with added aloin A compound, peak of aloin A is marked by aster

QUANTIFICATION

Table 4. Analysis of samples: Results (n = 3)

| Sample | compound (mg/100 ml) | | | | |
|--------------------------|----------------------|-----------|---------|---------|-------|
| | Quercetin | Hyperozid | Aloin A | Aloin B | Rutin |
| Pharmaceutical mixture 1 | 1.15 | 1.31 | 0.80 | / | 2.61 |
| Pharmaceutical mixture 2 | 0.89 | 1.35 | 6.82 | 7.46 | 2.75 |

