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Short communication

Determination of *trans*-resveratrol in human plasma by high-performance liquid chromatography

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Abstract

The first method using high-performance liquid chromatography (HPLC) has been developed for the determination of *trans*-resveratrol in human plasma. The method involves a liquid–liquid extraction followed by reversed-phase HPLC with UV detection. The detection limit of *trans*-resveratrol in human plasma was 5.0 ng/ml. Standard curves are linear over the concentration range of 5.0–5000.0 ng/ml. Intra-assay variability ranged from 1.9 to 3.7% and inter-assay variability ranged from 2.5 to 4.0% at the concentration range of 15.0–4000.0 ng/ml.

Keywords: *trans*-Resveratrol

1. Introduction

Resveratrol, 3,5,4′-trihydroxystilbene, which exists in both the *cis* and *trans* configurations, is found in oriental folk medicines and is reputed to benefit persons afflicted by a wide range of disorders including those affecting the liver, skin, heart, circulation, and lipid metabolism [1]. It was of no interest to the biochemical or clinical sciences until 1992, when Siemann and Creasy reported the presence of *trans*-resveratrol in red wine [2].

In recent years, more interest has been focused on *trans*-resveratrol [3–7]. Many researchers have shown that consumption of moderate amounts of red wine by human subjects is accompanied by increased blood anti-oxidant activity [8–11], which results in beneficial effects on human health.

Bertelli et al. [12,13] studied *trans*-resveratrol concentrations in rat plasma and in different organs after the administration of red wine. Up until now, although many publications have focused on the determination of *trans*-resveratrol concentrations in wine, few publications are available on the determination of *trans*-resveratrol concentrations in plasma. Blache et al. [14] described a gas chromatographic method for the determination of resveratrol concentrations in human plasma. The limit of quantitation was 50 ng/ml. Because of the increasing interest in *trans*-resveratrol, a method for determining the concentration of *trans*-resveratrol in human plasma was developed.

The method described here is the first HPLC method to quantify *trans*-resveratrol in human plasma using UV detection. This method involves a

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liquid/liquid extraction. It is simple, sensitive and reliable with a limit of quantitation of 5.0 ng/ml.

2. Experiments

2.1. Chemicals

Trans-resveratrol was obtained from Pharmatech (Fairfield, NJ, USA) and carbamazepine was purchased from Teva Pharmaceutical (Petach Tikva, Israel). Sodium phosphate monobasic, sodium phosphate dibasic, HPLC grade acetonitrile, ethyl acetate and methanol were purchased from Fisher Scientific (Nepean, Canada). HPLC grade water was obtained from an in-house Nano-pure water system.

The human plasma was obtained from Biological Specialty (Colmar, PA, USA). All plasma was stored at -20 ± 5°C.

2.2. Instrumentation

A Hewlett-Packard 1100 series HPLC system with an UV–Vis detector was used for this study. A 100×4.6 mm Inertsil Octyl/150 Å, 5 μm analytical column was purchased from Chromatography Sciences (St-Laurent, Canada). The detector was set at 310 nm. The mobile phase consisted of 30% acetonitrile, 70% 25 m*M* sodium phosphate mono-basic at pH 4.2 with a flow-rate of 1 ml/min.

2.3. Stock solutions and standards

Stock solutions of 100 μg/ml *trans*-resveratrol and 1.00 mg/ml carbamazepine (internal standard) were prepared in methanol. A seven non-zero calibration standard curve, ranging from 5.0 to 5000.0 ng/ml, was prepared by spiking the blank plasma with appropriate amounts of *trans*-resveratrol. The quality control (QC) samples (at three concentration levels) were prepared in a similar manner from a stock solution of *trans*-resveratrol (100 μg/ml). The quality control samples are extracted with the calibration standards to verify the integrity of the method.

2.4. Sample preparation

Internal standard working solution (100 μl of 20.0 μg/ml carbamazepine) and 0.25 *M* sodium phosphate dibasic buffer solution (250 μl) were added to spiked human plasma samples (500 μl). Ethyl acetate (6 ml) was then added, mixed with a shaker and then centrifuged for 10 min at 3000 rpm. The organic layer was transferred to a glass culture tube and dried with a nitrogen evaporator. The residue was reconstituted with 200 μl of mobile phase and 40 μl was then injected into the HPLC.

2.5. Calculations

The peak height ratio method (*trans*-resveratrol/internal standard) was used for quantitation. The *trans*-resveratrol concentration in the human plasma samples were determined using a standard curve analyzed by weighed linear regression (weighing factor $1/x^2$).

3. Results

Fig. 1 represents chromatograms of extracted plasma samples spiked with 1000.0 ng/ml *trans*-resveratrol, 5.0 ng/ml *trans*-resveratrol and a blank plasma. As can be seen, the extraction is very selective with no interference at the retention times of *trans*-resveratrol and internal standard.

3.1. Recovery

The absolute recovery was determined by comparing the peak height of extracted quality control samples prepared in human plasma, with the peak height of solutions prepared at the same concentrations. The analysis was done in six replicates at concentration levels of 15 and 4000 ng/ml. The recovery of *trans*-resveratrol from spiked plasma sample of 15 ng/ml was $102.1\pm3.4\%$ and the recovery was $96.7\pm6.7\%$ from a spiked plasma sample of *trans*-resveratrol at 4000 ng/ml. The recovery of the internal standard was found to be $100.9\pm5.7\%$.

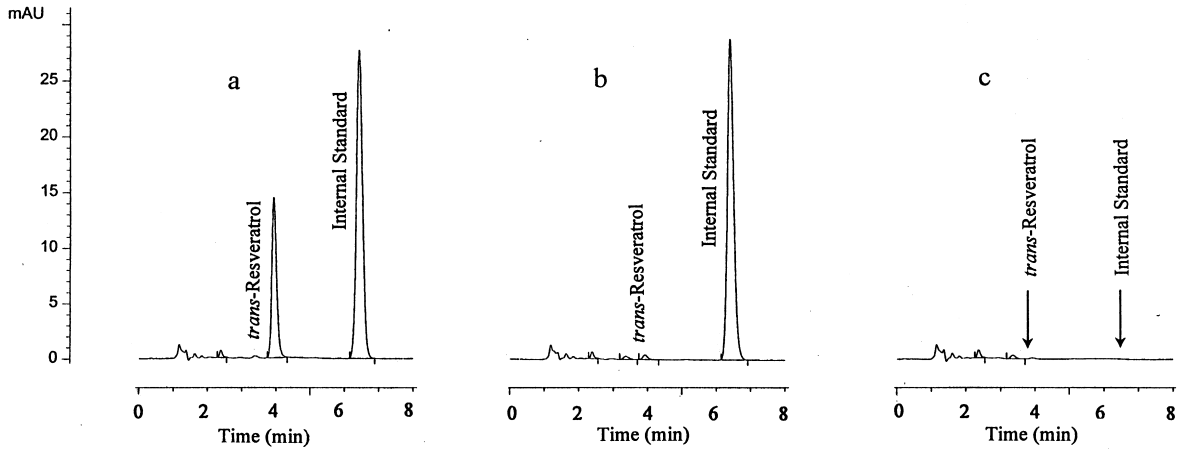

Fig. 1. (a) Chromatogram of extracted human plasma spiked with 1000 ng/ml *trans*-resveratrol, (b) Chromatogram of extracted LOQ (5.0 ng/ml *trans*-resveratrol), (c) Chromatogram of extracted blank plasma. Retention time for *trans*-resveratrol is 3.9 and 6.4 min for internal standard.

3.2. Limit of quantitation, linearity and precision

The results for the calibration samples are presented in Table 1. As can be seen, the calibration curves are linear in the concentration range of 5.0–5000.0 ng/ml. The regression was better than 0.9976 with a mean of 0.9989±0.0008 (n=5). The limit of quantitation (LOQ) for *trans*-resveratrol in human plasma was 5.0 ng/ml.

Inter-assay precision was determined by analyzing five calibration curves with quality control samples on five different days. The intra-assay precision was determined by analyzing replicates of quality control samples extracted in the same batch. The results of inter-day and intra-day precision are tabulated in Table 2. At the concentration range of 15.0–4000.0 ng/ml, the inter-assay and intra-assay coefficients of variation (C.V.'s) range from 2.5 to 4.0% and 1.9 to 3.7%, respectively.

4. Conclusions

The method described in this paper provides a simple and sensitive procedure for the quantitation of *trans*-resveratrol in human plasma samples. The method gives good linearity and reproducibility with quantitation limit of 5 ng/ml. This procedure has been applied to plasma samples from rabbit, rats and mice with minor modifications. The limit of quantitation from these animal plasma samples is 15–20 ng/ml. The difference in the limit of quantitation is

Table 1
Summary of calibration standards[a]

Concentration added (ng/ml)	Concentration found (ng/ml)	R.E.[b] (%)	C.V. (%)	n
5.00	4.91±0.03	−1.8	0.6	5
10.00	10.32±0.11	3.2	1.1	5
50.00	50.83±1.37	1.7	2.7	5
250.00	250.69±6.15	0.3	2.5	5
1000.00	1002.28±14.90	0.2	1.5	5
2500.00	2467.94±51.23	−1.3	2.1	5
5000.00	4884.08±166.31	−2.3	3.4	5

[a] Correlation coefficient=0.9989±0.0008 (n=5).
[b] R.E.=Relative error.

Table 2
Assay variability of *trans*-resveratrol in human plasma

Concentration added (ng/ml)	Concentration found (ng/ml)	R.E.[a] (%)	C.V. (%)	*n*
Inter-day				
15.00	14.51±0.58	−3.3	4.0	30
800.00	768.60±25.90	−3.9	3.4	10
4000.00	4013.14±99.75	0.3	2.5	29
Intra-day				
5.00	4.70±0.11	−6.0	2.5	6
15.00	14.26±0.52	−4.9	3.7	6
800.00	768.15±18.09	−4.0	2.4	2
4000.00	4015.04±76.73	0.4	1.9	6

[a] R.E.=Relative error.

attributed to the interference from unknown plasma components.

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