人血清中奎尼丁的薄层荧光光密度测定法

何 丽 一

(中国医学科学院药物研究所，北京)

测定人血清中抗心律不齐药物奎尼丁的浓度对确定合适的用药剂量及投药间隔时间十分重要。关于奎尼丁的血药浓度测定报道很多，有荧光法[1,2]、分光法[3]、气相色谱法[4]、高效液相色谱法[5]、薄层色谱法[6~9]、化学电离质谱法[10]以及酶免疫法[11]等。本文简化了提取方法，建立了薄层分离人血清中的奎尼丁后荧光光密度测定含量的方法；用本法分析人血清中奎尼丁的含量一次只需血清 50 μl，不同浓度的标准品回收率为 94.71～107.22%，变异系数在 5% 以内，血清中奎尼丁的荧光代谢产物不干扰测定，因此本法具灵敏、快速、重现性好及特异性强等优点，为临床测定奎尼丁的血药浓度提供了一个实用的方法。

材 料 和 方 法

(一) 药品、试剂及仪器

奎尼丁硫酸盐 BDH Laboratory reagent；氯仿、甲醇、甲酸均为分析纯。岛津 CS-910 双波长薄层扫描仪；岛津 C-EIB 数据处理机；微量毛细定量点样管 1 μl，5 μl。

(二) 样品制备

1. 标准溶液 精密称取奎尼丁硫酸盐 1.0 mg 于 2 ml 容量瓶中，用甲醇溶解并稀释至刻度，混匀后分别吸取 20，40，60 及 80 μl 于 1 ml 容量瓶中，用甲醇稀释至 1 ml，使成 10，20，30 及 40 ng/μl 的标准溶液。

2. 回收试验 取上述标准溶液各 10 μl 于四支 1 ml 带塞试管中，用冷风吹干甲醇，残渣中加 50 μl 空白血清，加氯仿 0.5 ml 振摇 3 min，离心后用微量注射器吸取氯仿提取液 400 μl 于另四支清洁的 1 ml 带塞试管中，于 80°C 以下水浴中蒸去氯仿，点样前将残渣溶于 40 μl 氯仿中。

3. 样品分析 精密吸取含奎尼丁的血清 50 μl 于 1 ml 带塞试管中，加氯仿 0.5 ml，振摇 3 min，离心后用微量注射器吸取氯仿提取液 400 μl 于另一清洁的 1 ml 带塞试管中，于 80°C 以下水浴中蒸去氯仿，点样前残渣溶于 40 μl 氯仿中。

(三) 薄层分离

将自制的硅胶 G 板(10×15 cm) 横向分割成 14 条宽度为 1 cm 的窄条，分别点标准溶液及给药后病人血清提取液一定量，用氯仿—甲醇 (9.2:0.8)预先饱和层离槽 20 min，再将点样后的薄层板置该层离槽中展开 8.5 cm，挥去展开剂后，薄层用甲酸蒸气熏 5～10 min，在紫外灯下奎尼丁在 Rf 值 0.52 处呈亮蓝色荧光。

(四) 含量测定

1. 点样 分别点不同浓度标准溶液各 1 μl，空白血清加不同量标准品提取液各 5 μl 或给药后病人血清氯仿提取液各 5 μl。

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2. 薄层分离　同前。

3. 光密度测定条件　光源　氙灯；测定方法　反射法直线扫描；波长　λex 350 nm，λem 450 nm；狭缝　0.5×10 mm；扫描速度　40 mm/min；纸速　20 mm/min。

4. 计算　空白血清加标准品及给药后病人血清的氯仿提取液，点样，分离后测得奎尼丁的面积值，用随行不同量奎尼丁标准品测得的面积值所制得的标准曲线计算回收率及样品中奎尼丁的血浓度。

结 果 与 讨 论

（一）如何从血清中定量提取奎尼丁，是测定奎尼丁血药浓度的关键之一，我们比较了几个从血清中提取奎尼丁的方法[6~9]，结果均不理想。本文利用奎尼丁硫酸盐在氯仿中易溶的特性，血清不需碱化，样品测定时只用 50 μl 血清加十倍量氯仿一次提取即可达到定量回收的目的；文献报道必须在低温下用氮气流除尽有机溶剂，我们比较了 80°C 以下加热及在室温下自然挥干氯仿提取液，测得结果一致，因此本法比文献报道的奎尼丁自血清中提出的方法具有样品用量少，提取方法简便及重现性好等特点。结果见表 1，2。

Tab 1. Recovery of quinidine in serum

Added (ng)	Determined (ng)	Recovery(%)	n
11.20	12.01	107.22	5
22.40	21.22	94.71	6
33.60	33.02	98.26	6
44.80	44.51	100.46	3

（二）薄层分离后观察到给药后病人血清中除奎尼丁外还有 2～4 个荧光代谢产物，因此薄层层离后用荧光光密度法可测定硫酸奎尼丁的原形药，方法具有很强的特异性，见图 1。

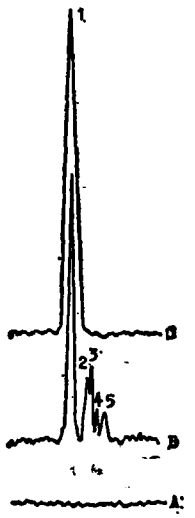

Fig 1. Thin layer chromatogram and densitometric scan of quinidine. A. Blank serum; B. Serum of patient given quinidine; C. Serum plus quinidine 1. Quinidine; 2,3,4,5. Metabolities of quinidine

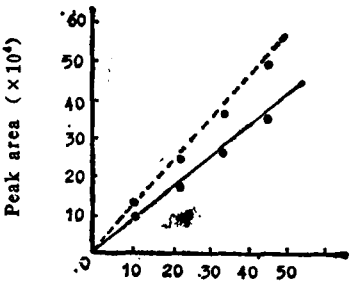

Fig 2. Linear range of quinidine
—— Standard curve of quinidine
---- Standard curve of quinidine after fumed with formic acid vapor

Tab 2. Comparisom of the method of removal of chloroform

Number of determination	Concentration of quinidine in serum (μg/ml)		CV%
	Room temperature	80°C	
1	1.40	1.45	
2	1.30	1.50	
3	1.45	1.50	
4	1.41	/	
Average	1.41	1.48	3.41

（三）奎尼丁薄层荧光光密度测定法的线性范围为 5～50 ng/点，用硫酸乙醇溶液喷奎尼丁斑点后可提高检测灵敏度[7]，但操作麻烦，而且不易喷雾均匀，我们将展开后的薄层置甲酸蒸气中 5 分钟，可提高灵敏度 40% 左右，见图 2。

（四）本法已用于临床，测定了给药后病人血清中奎尼丁的浓度，测得结果及临床分析将另文报道。

关键词 奎尼丁；血清；薄层分离；荧光光密度测定

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TLC SEPARATION AND FLUORODENSITOMETRIC DETERMINATION OF QUINIDINE IN HUMAN SERUM

HE Li-Yi

(*Institute of Materia Medica, Chinese Academy of Medical Sciences, Beijing*)

ABSTRACT A simple, sensitive, accurate and specific TLC fluorodensitometric method for the separation and determination of quinidine in human serum is developed.

Extraction: Fifty μl of serum is shaken with 0.5 ml of chloroform for 3 min in a 1 ml glass stoppered tube and then centrifuged for 3 min at 2000 r/min. Four hundred μl of chloroform layer is transferred to another tube and evaporated to dryness below 80°C in a water bath. The residue is dissolved in 40 μl of chloroform.

Chromatography: Glass plates (10×15 cm) are coated with silica gel G and air dried. Equally spaced vertical grooves are traced through the adsorbent layer to divide it into 14 strips each 1.0 cm wide. Five μl aliquots of the extract are applied at points about 1.5 cm from the bottom edge of the plate, and the chromatogram is developed in a suitable chamber using chloroform—methanol (9.2:0.8) as the developing solvent. Until the solvent front has ascended about 8.5 cm from the point of application, quinidine and its metabolites are thus separated. The plate is removed, air-dried, and the chromatogram is fumed with formic acid vapor, quinidine can be seen as light blue fluoroscent spots under UV light.

Determination: Quinidine spots are measured fluorometrically using an excitation wavelength of 350 nm and a 450 nm filter for the emission wavelength. The results are calculated by external standard method. A linear relationship is obtained for quinidine in the range of 5～50 ng. The recovery from serum is from 94.71～107.22% for quinidine.

The method is recommended for clinical assay and pharmacokinetic studies.

Key words Quinidine; TLC separation; Fluorodensitometric determination