RNA Chromatography Under Thermally Denaturing Conditions: Quantitation of RNA

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Chromatography of RNA at elevated denaturing temperatures using the WAVE™ Nucleic Acid Fragment Analysis System and an RNASep™ Cartridge is presented as a safe and reliable method for RNA quantitation.

Introduction

Quantitation of RNA samples is currently performed by measuring the absorbance of an RNA solution at 260 nm using a UV spectrophotometer, or by gel electrophoresis followed by ethidium bromide staining and visualization on a UV light box, or by Northern blot analysis (1). Quantitating an RNA sample by measuring its UV absorbance, however, does not provide any information about the integrity of the RNA sample, e.g. presence of impurities or degradation. The quality and quantity of the RNA analyzed by gel electrophoresis is jeopardized by the presence of ribonucleases in the analysis buffer, the gel and the electrophoresis apparatus. The RNA analyzed cannot be recovered and used in downstream applications.

Here, we present data on a faster, safer, less labor intensive, automated and highly accurate alternative method for RNA analysis. RNA chromatography using the WAVE Nucleic Acid Fragment Analysis System with the RNASep Cartridge provides a means for reliable RNA quantitation. This method combines the high resolving capability of RNA chromatography with the highly automated sampling and data acquisition functions of WAVEMAKER™ Software. Each RNA peak can be quantitated by peak integration (performed automatically by WAVEMAKER Software) and purified by peak capture. Since the RNA collected by peak capture was found to be more stable than the original sample applied, it is well suited for subsequent investigations (2-4).

Materials and Methods

The WAVE System equipped with an RNASep Cartridge (internal diameter of 7.8 mm and a length of 50 mm) and triethyl-ammonium acetate (TEAA) were provided by Transgenomic, Inc. (Omaha NE). MS2 RNA (from bacteriophage MS2) and TMV RNA (from tobacco mosaic virus) were purchased from Roche Molecular Biochemicals (Indianapolis, IN). Standard buffers used were (A) 0.1 M TEAA and (B) 0.1 M TEAA, 25 % acetonitrile (ACN), each at pH 7.0.

RNA chromatography was carried out under denaturing conditions at 75°C using the WAVE System and the RNASep Cartridge. The stationary phase in the RNASep Cartridge consists of a nonporous alkylated poly(styrene-divinylbenzene) bead matrix. The mobile phase consists of a two-eluant buffer system composed of buffer A and buffer B. Buffer A consists of 0.1 M triethylammonium acetate (TEAA), pH 7.0, and buffer B consists of 0.1 M TEAA at pH 7.0 containing 25% by volume acetonitrile (ACN). Gradient conditions applicable to Figures 1 and 3 are presented in Table 1. Gradient conditions applicable to Figure 4 are presented in Table 2.

RNA eluting from the column was detected with a UV detector at 260 nm, and the data were recorded and displayed as chromatograms by the WAVE System's WAVEMAKER Software. RNA amounts were quantitated by peak integration using WAVEMAKER Software and RNA peaks were collected manually by peak capture.

Results and Discussion

MS2 RNA marker, 3569 nts, was analyzed and quantitated on the WAVE System. RNA was diluted in DEPC-treated water (1) to produce five different concentrations (0.8 ng/µL, 4.0 ng/µL, 12.5 ng/µL, 50 ng/µL and 200 ng/µL). Different amounts of MS2 RNA were injected and analyzed on the WAVE System as shown in Figures 1a and 1b. For double-stranded DNA samples, using a flow rate of 0.9 mL/min, 1000 mVs of the WAVE System's calculated peak area is approximately equivalent to 1 ng of DNA. For RNA about 1250 mVs of calculated peak area is equivalent to 1 ng of RNA ($1A_{260}$ of double-stranded DNA = 50 µg/mL, $1A_{260}$ of single-stranded RNA = 40 µg/mL, which gives a ratio of 50/40 or a factor of 1.25). The experimental procedure for determining the correct correlation factor is described in

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Differences between calculated and measured RNA concentrations decrease as the amounts of handled RNA increase. Therefore, for low concentrations of RNA, a standard curve should be generated to more precisely determine the concentration of RNA samples. A standard curve for MS2 RNA quantitation results is plotted in Figure 2. In addition to RNA quantitation, the WAVE System

provides a powerful visual way of assessing RNA integrity, purity and stability (5). In Figures 1a and 1b, a well-resolved peak for MS2 RNA shows the degree of purity and stability of this RNA sample. However, when RNA samples undergo degradation by ribonucleases or chemical hydrolysis, they break down into smaller fragments, which have a weaker interaction with the RNASep Cartridge and thus elute faster. This is shown in Figure 1b in which some RNA degradation is observed (smaller peaks appearing to the left of the 3569-nt peak) for the higher concentrations of MS2 RNA injected.

In Figure 3, 0.50 µg of TMV RNA (6,350 nts) was injected and quantitated on the WAVE System. The injected and calculated amounts (0.54 µg) of TMV RNA are very similar. In Figure 4, a very low concentration of an RNA transcript is quantitated. The concentration of this RNA sample is 0.22 ng/µL; for a 10-µL injection volume the peak corresponds to 2.2 ng of RNA. The low concentration and the quality of this RNA sample cannot be assessed by gel electrophoresis techniques.

In these experiments, analysis of RNA samples is fully automated and can be achieved in minutes. Since RNA detection is performed by UV spectroscopy at 260 nm there is no need for the use of harmful chemicals such as ethidium bromide. Furthermore, RNA samples being analyzed and quantitated can simultaneously be purified by peak capture for use in downstream applications such as cDNA library construction, *in situ* hybridization analysis and cDNA arrays. RNA is especially stable during and after analysis on the WAVE System (5, 6). The WAVE System, therefore, allows a fast, accurate, safe and automated alternative to the classical methods of RNA analysis.

Conclusions

The WAVE System is a powerful alternative method of RNA analysis. The advantages are the high sensitivity of on-line UV detection (as low as 2 ng per peak) coupled with the high-resolution capability of RNA chromatography, as well as the ease of recovery of stabilized RNA samples for subsequent studies.

Table 1

Gradient Conditions for Figures 1 and 3				
Time (minutes)	**Buffer A (%)**	**Buffer B (%)**	**Flow Rate (ml/min)**	**Temperature (°C)**
0	62	38	0.90	75
1	60	40	---	---
16	40	60	---	---
22	34	66	---	---
22.5	30	70	---	---
23	0	100	---	---
24	0	100	---	---
25	62	38	---	---
27	62	38	---	---

Table 2

Gradient Conditions for Figure 4				
Time (minutes)	**Buffer A (%)**	**Buffer B (%)**	**Flow Rate (ml/min)**	**Temperature (°C)**
0	100	0	0.90	75
30	30	70	---	---
31	0	100	---	---
33	100	0	---	---

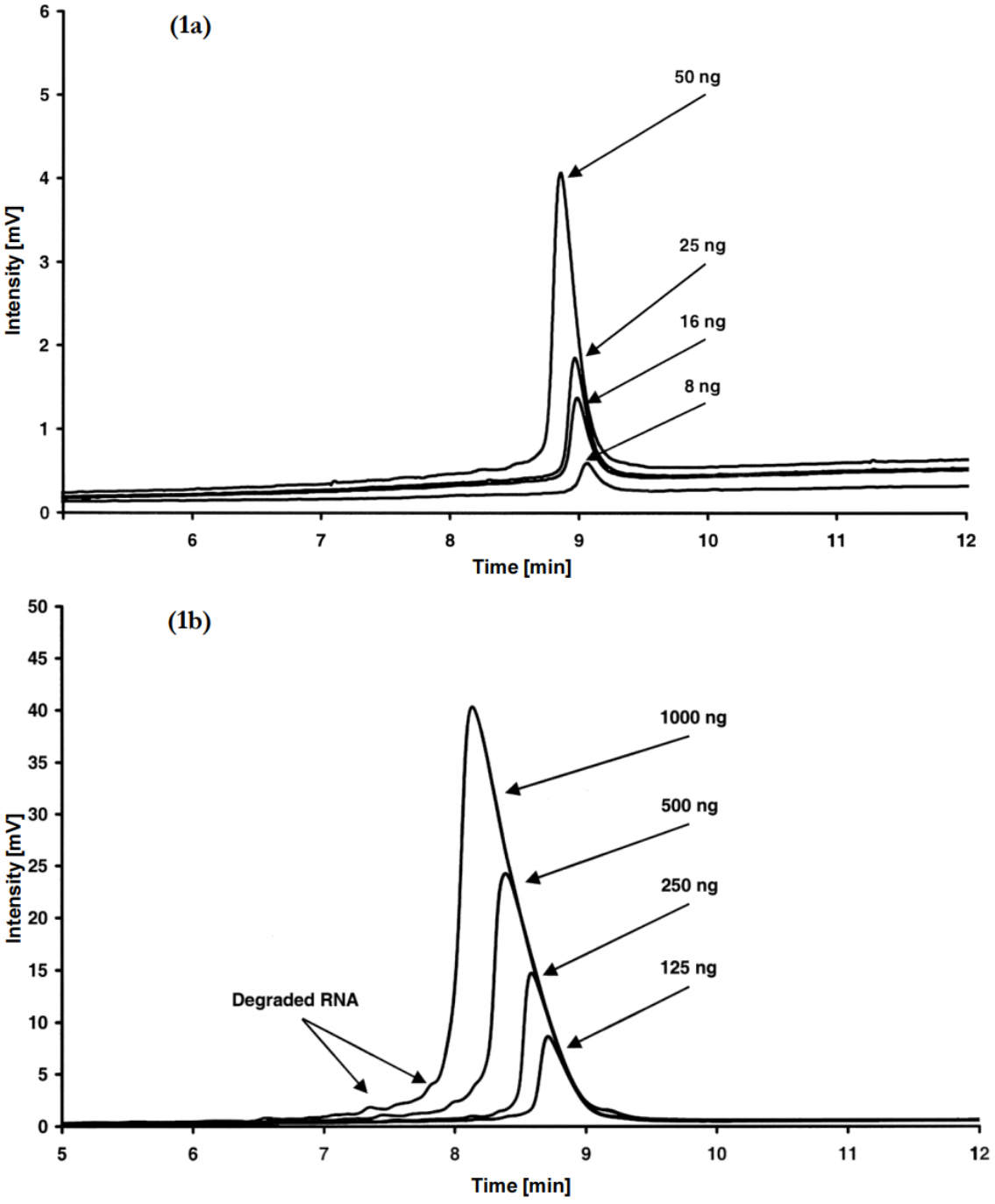

Figure 1: MS2 RNA Quantitation on the WAVE System
MS2 RNA was diluted to yield five different concentrations: 0.8ng/µL, 4.0 ng/µL, 12.5ng/µL, 50ng/µL, and 200ng/µL.

(a) Injections of 10µL of the 0.8ng/µL solution, 4µL of the 4.0ng/µL solution, 2µL of the 12.5ng/µL solution, and 4µL of the 12.5ng/µL solution.
(b) Injections of 10µL of the 12.5ng/µL solution, 5µL of the 50ng/µL solution, 10µL of the 50ng/µL solution, and 5µL of the 200ng/µL solution.

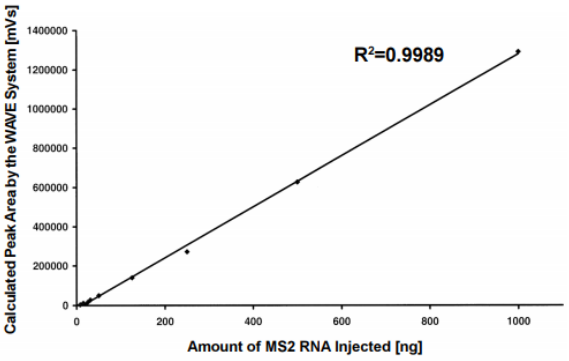

Figure 2: RNA Quantitation Standard Curve
Standard curve generated using the MS2 RNA quantitation results.

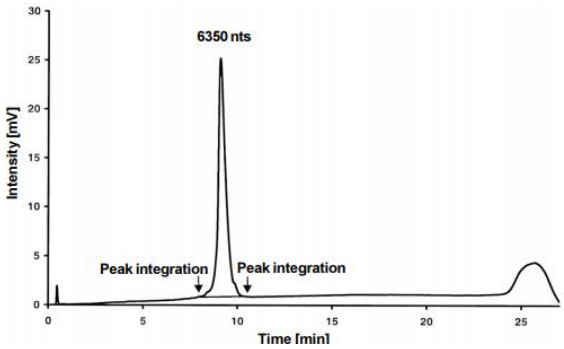

Figure 3: TMV RNA (0.50µg) was analyzed and quantitated on the WAVE System.
The calculated peak area is equivalent to 0.54µg of RNA.

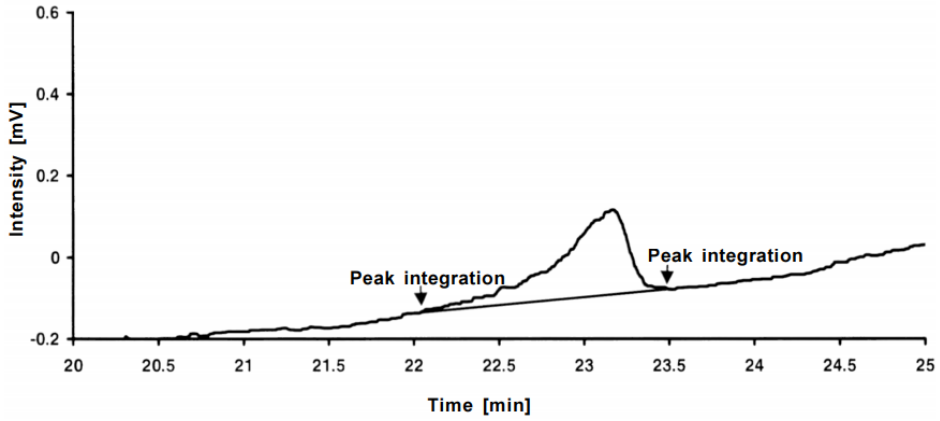

Figure 4: RNA transcript (10µL) analyzed and quantitated on the WAVE System.
The calculated peak area corresponds to 2.2ng of RNA (0.22ng/µL)

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