

B. GENERAL TESTS

Ion Chromatography

Ion chromatography is designed to analyze individual components in a mixture, using a column packed with a suitable material, such as an ion exchanger as a stationary phase and an eluent as a mobile phase. A test sample injected into the column is separated into individual components by flowing the eluent through the column. This method utilizes the difference in ion-exchange capacity of individual components. This method is applicable to liquids or substances which can be made into solutions, and is usually used for identification tests, purity tests, assays and other tests.

Apparatus The apparatus consists generally of a pump to move eluent, a sample injection device, a column, a detector, a recording system. The column is maintained at a constant temperature with equipment such as thermostat. The pump delivers the eluent into the column and the connection tubes and related devices at a constant flow rate.

The detector detects components which are different in property from the eluent and gives signals in proportion to the concentration for a substance of a few micrograms or less. Electric conductometers and UV spectrophotometers are usually used.

The recording system records the intensities of the signals obtained by the detector. When an electrical conductometer is used as the detector, a suppressor can be placed in front of the conductometer. The suppressor is used to reduce the electric conductivity of the eluent and amplifies the ratio of the noises to the signals.

Procedure Condition the apparatus previously, adjust the eluant, the column, the detector, the flow rate of the eluent to the operating conditions specified in the individual monograph, equilibrate the column at a specified temperature. Inject the test solution, the standard solution, or the control solution, prepared as specified in the individual monograph, into the sample injection device using a microsyringe or sample valve. Detect the separated components using the detector, and record the chromatogram on the recorder. Identification of the substance is carried out by confirming that the same retention time as for the standard solution is obtained, or that the retention time does not change nor does the peak width widen when the standard sample is added.

Determination is usually performed by method (1) or (2), using the peak height or peak area.

(1) Internal Standard Method Prepare several standard solutions containing a constant amount of the specified internal standard and known, graded amount of the standard object component. For each of the chromatograms obtained by injecting a

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constant volume of each standard solution, calculate the ratio of the peak height and peak area of the standard object component to that of the internal standard. Prepare a calibration curve by plotting these ratios on the ordinate and the ratios of each amount of the standard object component to the amount of the internal standard, or the amounts of the standard object component on the abscissa. The calibration curve is usually a straight line through the origin. Then, prepare the test solution containing the same amount of the internal standard as specified in the individual monograph, record its chromatogram under the same conditions as for the preparation of the calibration curve, calculate the ratio of the peak height or peak area of the object component to that of the internal standard, and perform the determination of the object component using the calibration curve.

(2) Absolute Calibration Curve Method Prepare several standard solutions containing graded amount of the standard object component, and inject a constant volume of each standard solution, accurately measured. For the chromatograms obtained, prepare a calibration curve by plotting the peak heights or peak areas of the standard object component on the ordinate and the amounts of the standard object component on the abscissa. The calibration curve is usually a straight line through the origin. Then, prepare the test solution as specified in the individual monograph, record its chromatogram under the same conditions as for the preparation of the calibration curve, and measure the peak height or peak area of the object component. Determine the object component using the calibration curve.

Unless there is any problem, when an anion standard solution is prepared, use its sodium or potassium salt, and when a cation standard solution is prepared, use its chloride or nitrate.

For either method above, the peak height or peak area is measured generally using an appropriate one of methods (1) and (2).

(1) Peak Height Use either of the two methods.

Peak height method Measure the distance between the peak maximum and the intersection of a perpendicular line drawn from the peak maximum to the horizontal axis of recording paper and a line linking the both side inflection points of the lower end of the peak.

Automatic peak height method Measure the signal from the detector and determine the peak height, using a data processor system.

(2) Peak Area Use either of the two methods.

Width at half-height method Multiply the peak width at half-height by the peak height.

Automatic integration method Measure the signal from the detector and determine the peak area, using a data processing system.