

## B. GENERAL TESTS

### Gas Chromatography

Gas Chromatography is designed to analyze individual components in a mixture, using a column made of a suitable immobile phase and a gas (carrier gas) as a mobile phase. Test sample injected into the column is developed in a gas state, and its individual components are separated and analyzed, using the difference in the retention capacity of individual components in the immobile phase. This method is applicable to gases, liquids, or vaporizable samples. The method is used for identification tests, purity tests, and assays.

**Apparatus** The apparatus consists generally of a carrier gas introducing port, a sample injection device, a column built in a thermostat, a detector, and a recorder. The carrier gas introducing port delivers a carrier gas at a constant rate to the column. The detector is a device to detect components with properties different from the carrier gas. Electric conductometers, flame-ionization detectors, and electron-capture detectors are generally used. The recorder is a device to record intensity of the signal obtained by the detector.

**Procedure** Unless otherwise specified, proceed as follows: Condition the apparatus previously and adjust the detector, the column, the temperature, and the flow rate of the carrier gas to the operating conditions specified in the individual monograph. Inject the test solution or the standard solution or control solution, prepared as specified in the individual monograph, into the sample injection device, using a microsyringe. Detect the separated components by the detector, and obtain a chromatogram recorded by the recorder.

The identification of the test substance is carried out by confirming that the same retention time (hereinafter in the publication, retention time refers to the time from the injection of the test solution to the emergence of the peak maximum of the component) 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 performed according to either of the following methods, generally 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 amounts of the standard object component. With each of the chromatograms obtained by injecting a constant volume of each standard solution, calculate the ratio of the peak height or peak area of the standard objection component to that of the internal

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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 directed in the individual monograph, record a 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, using the calibration curve.

(2) Absolute Calibration Curve Method Prepare standard solutions containing graded amount of the standard object component, and inject a constant volume of each standard solution, exactly measured. With 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 directed in the individual monograph, record a chromatogram under the same conditions as for the preparation of the calibration curve, measure the peak height or peak area of the object component, and perform the determination, using the calibration curve.

For either method above, the peak height or peak area is measured generally by 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 obtained from the detector, using a data processor device and determine the peak height.

(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 obtained from the detector, using a data processor device and determine the peak area.